

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : A61K 38/17, 31/70, 39/395, C12N 15/11, C12Q 1/68, G01N 33/50, 33/574, A01K 67/027, C12N 15/00</p>	A2	<p>(11) International Publication Number: WO 98/35693</p> <p>(43) International Publication Date: 20 August 1998 (20.08.98)</p>
<p>(21) International Application Number: PCT/IB98/00781</p> <p>(22) International Filing Date: 13 February 1998 (13.02.98)</p> <p>(30) Priority Data: 08/800,929 13 February 1997 (13.02.97) US</p> <p>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 08/800,929 (CIP) Filed on 13 February 1997 (13.02.97)</p> <p>(71) Applicant (for all designated States except US): UNIVERSITY OF OTTAWA [CA/CA]; 650 Cumberland, Ottawa, Ontario K1N 6N5 (CA).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): KORNELUK, Robert [CA/CA]; 1901 Tweed Avenue, Ottawa, Ontario K1G 2L8 (CA). MACKENZIE, Alexander, E. [CA/CA]; 35 Rockcliffe Way, Ottawa, Ontario K1M 1A3 (CA). LISTON, Peter [CA/CA]; 1 Second Avenue, Ottawa, Ontario K1S 2H2 (CA). BAIRD, Stephen [CA/CA]; 20 Julian Avenue, Ottawa, Ontario K1Y 0S5 (CA). TSANG, Benjamin [CA/CA];</p>	<p>1053 Carling Avenue, Ottawa, Ontario K1Y 4E9 (CA). PRATT, Christine [CA/CA]; 31 Long Gate Court, Nepean, Ontario K2J 4E7 (CA).</p> <p>(74) Agent: DEETH WILLIAMS WALL; National Bank Building, Suite 400, 150 York Street, Toronto, Ontario M5H 3S5 (CA).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>	
<p>(54) Title: DETECTION AND MODULATION OF THE IAPS AND NAIP FOR THE DIAGNOSIS AND TREATMENT OF PROLIFERATIVE DISEASE</p>		
<p>(57) Abstract</p> <p>Disclosed are diagnostic and prognostic methods and kits for the detection and treatment of proliferative diseases such as cancer (e.g., ovarian cancer, breast cancer, and lymphoma). Also disclosed are therapeutics for treating proliferative diseases (and methods for identifying such therapeutics) that utilize IAP and NAIP antisense nucleic acid molecules, antibodies which specifically bind IAP and NAIP polypeptides, and compounds that reduce the biological activities of IAP and NAIP polypeptides.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China			PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LJ	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Both normal cell types and cancer cell types display a wide range of susceptibility to apoptotic triggers. Many normal cell types undergo temporary growth arrest in response to a sub-lethal dose of radiation or cytotoxic chemical, while cancer cells in the vicinity undergo apoptosis. This provides the crucial treatment "window" of appropriate toxicity that allows successful anti-cancer therapy. It is therefore not surprising that resistance of tumor cells to apoptosis is emerging as a major category of cancer treatment failure. Finding compounds which overcome or prevent this resistance would greatly improve cancer therapies.

30 We have discovered that IAP and NAIP overexpression are specifically associated
with a wide range of cancer types including ovarian cancer, adenocarcinoma, lymphoma, and

- 2 -

pancreatic cancer. The presence of a fragmented IAP polypeptide in the nucleus, and an overexpression of an IAP polypeptide in the presence of a p53 mutation correlates with a cancer diagnosis, a poor prognosis, and a resistance to numerous chemotherapeutic cancer drugs. In addition, we have found that a therapeutic agent that reduces the biological activity of an IAP polypeptide will induce apoptosis in a cell expressing the polypeptide (e.g., a cell that is proliferating in a proliferative disease). These discoveries provide diagnostic and prognostic methods for the detection and treatment of proliferative diseases, and provide therapeutic compounds useful for the treatment of proliferative diseases, particularly cancer.

In a first aspect, the invention features a method for enhancing apoptosis in a cell from a mammal with a proliferative disease, the method including administering to the cell a compound that inhibits the biological activity of an IAP polypeptide or a NAIP polypeptide, the compound being administered to the cell in an amount sufficient to enhance apoptosis in the cell. In one embodiment of this aspect of the invention, the cell is proliferating in the proliferative disease. In another embodiment, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of polypeptide present in the cell); the level of expression of an mRNA molecule encoding the polypeptide; or an apoptosis-inhibiting activity.

In various embodiments of the first aspect of the invention, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiment, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2. In other preferred embodiments, the mammal is a human or a mouse, and the proliferative disease is cancer, for example, a cancer in a tissue selected from the group consisting of ovary, breast, pancreas, lymph node, skin, blood, lung, brain, kidney, liver, nasopharyngeal cavity, thyroid, central nervous system, prostate, colon, rectum, cervix, endometrium, and lung.

In various preferred embodiments of the first aspect of the invention, the compound is a negative regulator of an IAP or an NAIP-dependent anti-apoptotic pathway; a fragment of the IAP polypeptide, the fragment including a ring zinc finger and having no more than two BIR domains; a nucleic acid molecule encoding a ring zinc finger domain of the IAP polypeptide; a compound that prevents cleavage of the IAP polypeptide or the NAIP polypeptide; a purified antibody or a fragment thereof that specifically binds to the IAP polypeptide or the NAIP polypeptide; a ribozyme; or an antisense nucleic acid molecule have

- 3 -

a nucleic acid sequence that is complementary to the coding strand of a nucleic acid sequence encoding the IAP polypeptide or the NAIP polypeptide. Preferably, the cleavage is decreased by at least 20% in the cell; the antibody binds to a BIR domain of the IAP polypeptide or the NAIP polypeptide; the nucleic acid sequence encoding the IAP polypeptide or the NAIP polypeptide has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13; or the nucleic acid sequence of NAIP; the antisense nucleic acid molecule decreases the level of the nucleic acid sequence encoding the IAP polypeptide or the NAIP polypeptide by at least 20%, the level being measured in the cytoplasm of the cell; the antisense nucleic acid molecule is encoded by a virus vector; or the antisense nucleic acid molecule is encoded by transgene.

In a second aspect, the invention features a method for detecting a proliferative disease or an increased likelihood of the proliferative disease in a mammal that includes: (a) contacting an IAP or a NAIP nucleic acid molecule that is greater than about 18 nucleotides in length with a preparation of nucleic acid from a cell of the mammal, the cell proliferating in the disease, the cell from a tissue; and (b) measuring the amount of nucleic acid from the cell of the mammal that hybridizes to the molecule, an increase in the amount from the cell of the mammal relative to a control indicating an increased likelihood of the mammal having or developing a proliferative disease. In one embodiment, the method further includes the steps of: (a) contacting the molecule with a preparation of nucleic acid from the control, wherein the control is a cell from the tissue of a second mammal, the second mammal lacking a proliferative disease; and (b) measuring the amount of nucleic acid from the control, an increase in the amount of the nucleic acid from the cell of the mammal that hybridizes to the molecule relative to the amount of the nucleic acid from the control indicating an increased likelihood of the mammal having or developing a proliferative disease.

In one embodiment of the methods of the second aspect of the invention, the method further includes the steps of: (a) providing a pair of oligonucleotides having sequence identity to or being complementary to a region of the IAP or the NAIP nucleic acid molecule; (b) combining the pair of oligonucleotides with the nucleic acid under conditions suitable for polymerase chain reaction-mediated nucleic acid amplification; and (c) isolating the

- 4 -

amplified nucleic acid or fragment thereof. Preferably, the amplification is carried out using a reverse-transcription polymerase chain reaction (*e.g.*, RACE).

In one embodiment of the second aspect of the invention, the method provides measuring the nucleic acid having a nucleotide sequence that has about 50% or greater
5 identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or the nucleic acid sequence of NAIP. In other embodiments, the method provides measuring the nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, or NAIP.

10 In a third aspect, the invention features a method for detecting a proliferative disease or an increased likelihood of developing the disease in a mammal, the method including measuring the level of biological activity of an IAP polypeptide or a NAIP polypeptide in a sample of the mammal, an increase in the level of the IAP polypeptide or the NAIP polypeptide relative to a sample from a control mammal being an indication that the mammal
15 has the disease or increased likelihood of developing the disease. In various embodiments, the sample includes a cell that is proliferating in the disease from the mammal, the cell from a tissue; and the sample from a control mammal is from the tissue, the sample consisting of healthy cells. In another embodiment, the mammal and the control mammal are the same.

In various embodiments of the third aspect of the invention, the biological activity is
20 the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other
25 embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a fourth aspect, the invention features a method for identifying a compound enhances apoptosis in an affected cell that is proliferating in a proliferative disease that includes exposing a cell that overexpresses an IAP polypeptide or a NAIP polypeptide to a candidate compound, a decrease the level of biological activity of the polypeptide indicating
30 the presence of a compound that enhances apoptosis in the affected cell that is proliferating in the proliferative disease.

- 5 -

In a fifth aspect, the invention features a method for identifying a compound that enhances apoptosis in an affected cell that is proliferating in a proliferative disease that includes the steps of: (a) providing a cell including a nucleic acid molecule encoding a IAP polypeptide or a nucleic acid molecule encoding a NAIP polypeptide, the nucleic acid molecule being expressed in the cell; and (b) contacting the cell with a candidate compound and monitoring level of biological activity of the IAP polypeptide or the NAIP polypeptide in the cell, a decrease in the level of biological activity of the IAP polypeptide or the NAIP polypeptide in the cell in response to the candidate compound relative to a cell not contacted with the candidate compound indicating the presence of a compound that enhances apoptosis in the affected cell that is proliferating in the proliferative disease. Preferably, the cell further expresses a p53 polypeptide associated with the proliferative disease.

In various embodiments of the fourth and fifth aspects of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a sixth aspect, the invention features a method for determining the prognosis of a mammal diagnosed with a proliferative disease that includes the steps of: (a) isolating a sample from a tissue from the mammal; and (b) determining whether the sample has an increased an level of biological activity of an IAP polypeptide or an NAIP polypeptide relative to a control sample, an increase in the level in the sample being an indication that the mammal has a poor prognosis. In various embodiments of this aspect of the invention, the sample includes a cells that is proliferating in the proliferative disease and the control sample is from the tissue, the control sample consisting of healthy cells; and the sample and the control sample are from the mammal. Preferably, the sample further includes a cell expressing a p53 polypeptide associated with the proliferative disease.

In various embodiments of the sixth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression

- 6 -

of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2. In a preferred
5 embodiment, the level is assayed by measuring the amount of IAP peptide of less than 64 kDa present in the sample.

In a seventh aspect, the invention features a method for determining the prognosis of a mammal diagnosed with a proliferative disease that includes the steps of: (a) isolating a sample from the mammal, the sample having a nuclear fraction; and (b) measuring the
10 amount of a polypeptide that is recognized by an antibody that specifically binds an IAP polypeptide or an antibody that specifically binds an NAIP polypeptide in the nuclear fraction of the sample relative an amount from a control sample, an increase in the amount from the sample being an indication that the mammal has a poor prognosis. In preferred embodiments of this aspect of the invention, the sample is from a tissue of the mammal, the
15 sample including a cell that is proliferating in the proliferative disease, and the control sample is from the tissue, the control sample consisting of healthy cells. In another embodiment, the sample and the control sample are from the mammal.

In various embodiments of the seventh aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount
20 of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2. In
25 another embodiment, the amount is measured by immunological methods.

In an eighth aspect, the invention features a method for treating a mammal diagnosed as having a proliferative disease that includes the steps of: (a) measuring the amount of an IAP or NAIP polypeptide in a first sample from a tissue from the mammal, the first sample including a cell that is proliferating in the proliferative disease; (b) measuring the amount of
30 the polypeptide in a second sample from the tissue, the second sample consisting of healthy cells; (c) detecting an increase in the amount of the polypeptide in the first sample to the

- 7 -

amount of the polypeptide in the second sample; and (d) treating the mammal with a compound that decreases the biological activity of the polypeptide. Preferably, the first sample and the second sample are from the mammal.

In various embodiments of the eighth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other
10 embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a ninth embodiment, the invention features the use of a compound that decreases the biological activity an IAP polypeptide or a NAIP polypeptide for the manufacture of a medicament for the enhancement of apoptosis.

In various embodiments of the ninth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other
20 embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a tenth aspect, the invention features a kit for diagnosing a mammal for the presence of a proliferative disease or an increased likelihood of developing a proliferative disease, the kit comprising an oligonucleotide that hybridizes to a nucleic acid sequence that encodes an IAP polypeptide or a NAIP polypeptide.

25 In various embodiments of the tenth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other
30 embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

- 8 -

In an eleventh aspect, the invention features a transgenic mammal, the mammal having an elevated level of biological activity of an IAP polypeptide or a NAIP polypeptide.

In various embodiments of the eleventh aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount
5 of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

10 By "IAP gene" is meant a gene encoding a polypeptide having at least one BIR domain and is capable of modulating (inhibiting or enhancing) apoptosis in a cell or tissue when provided by other intracellular or extracellular delivery methods (see, *e.g.*, the U.S.S.N.s 08/511,485, 08/576,965, and PCT/IB96/01022). In preferred embodiments the IAP gene is a gene having about 50% or greater nucleotide sequence identity to at least one
15 of the IAP amino acid encoding sequences of Figs. 1-6 (SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13) or portions thereof, or has a ring zinc finger domain. Preferably, the region of sequence over which identity is measured is a region encoding at least one BIR domain and a ring zinc finger domain. Mammalian IAP genes include nucleotide sequences isolated from any mammalian source. Preferably, the
20 mammal is a human. The term "IAP gene" is meant to encompass any member of the family of genes that encode inhibitors of apoptosis. An IAP gene may encode a polypeptide that has at least 20%, preferably at least 30%, and most preferably at least 50% amino acid sequence identity with at least one of the conserved regions of one of the IAP members described herein (*i.e.*, either the BIR or ring zinc finger domains from the human or murine XIAP,
25 HIAP-1, or HIAP-2). Representative members of the IAP gene family include, without limitation, the human and murine XIAP, HIAP-1, or HIAP-2 genes.

By "a virus vector" is meant a functional or attenuated virus that is capable of delivering to a virus-infected cell a nucleic acid molecule. Preferably, the virus vector has been genetically engineered according to standard molecular biology techniques to bear a

- 9 -

heterologous nucleic acid molecule. Virus vectors include, without limitation, adenoviruses, retroviruses, baculoviruses, cytomegaloviruses (CMV), and vaccinia viruses.

By "IAP protein" or "IAP polypeptide" is meant a polypeptide, or fragment thereof, encoded by an IAP gene.

5 By "NAIP gene" and "NAIP polypeptide" is meant the NAIP genes, fragments thereof, and polypeptides encoded by the same described in UK9601108.5 filed January 19, 1996 and PCT Application No. PCT/IB97/00142 (claiming priority from UK9601108.5) filed January 17, 1997.

By "BIR domain" is meant a domain having the amino acid sequence of the
 10 consensus sequence: Xaal-Xaal-Xaal-Arg-Leu-Xaal-Thr-Phe-Xaal-Xaal-Trp-Pro-Xaa2-Xaal-Xaal-Xaa2-Xaa2-Xaal-Xaal-Xaal-Xaal-Leu-Ala-Xaal-Ala-Gly-Phe-Tyr-Tyr-Xaal-Gly-Xaal-Xaal-Asp-Xaal-Val-Xaal-Cys-Phe-Xaal-Cys-Xaal-Xaal- Xaal-Xaal-Xaal-Xaal-Trp-Xaal-Xaal-Xaal-Asp-Xaal-Xaal-Xaal- Xaal-Xaal-His-Xaal-Xaal-Xaal-Xaal-Pro-Xaal-Cys-Xaal-Phe-Val, wherein Xaal is any amino acid and Xaa2 is any amino acid or is absent (SEQ ID
 15 NO: 2). Preferably, the sequence is substantially identical to one of the BIR domain sequences provided for XIAP, HIAP-1, or HIAP-2 herein.

By "ring zinc finger" or "RZF" is meant a domain having the amino acid sequence of the consensus sequence: Glu-Xaal-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa2-Xaal-Xaal-Xaal-Cys-Lys-Xaa3-Cys-Met-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa3-Xaal-Phe-Xaal-Pro-Cys-Gly-His-Xaal-
 20 Xaal-Xaal-Cys-Xaal-Xaal-Cys-Ala- Xaal-Xaal-Xaal-Xaal-Xaal-Cys-Pro-Xaal-Cys, wherein Xaal is any amino acid, Xaa2 is Glu or Asp, and Xaa3 is Val or Ile (SEQ ID NO:1).

Preferably, the sequence is substantially identical to the RZF domains provided herein for the human or murine XIAP, HIAP-1, or HIAP-2.

By "enhancing apoptosis" is meant increasing the number of cells which apoptose in
 25 a given cell population. Preferably, the cell population is selected from a group including ovarian cancer cells, breast cancer cells, pancreatic cancer cells, T cells, neuronal cells, fibroblasts, or any other cell line known to proliferate in a laboratory setting. It will be appreciated that the degree of apoptosis enhancement provided by an apoptosis enhancing compound in a given assay will vary, but that one skilled in the art can determine the
 30 statistically significant change in the level of apoptosis which identifies a compound which enhances apoptosis otherwise limited by an IAP. Preferably, "enhancing apoptosis" means

- 10 -

that the increase in the number of cells undergoing apoptosis is at least 25%, more preferably the increase is 50%, and most preferably the increase is at least one-fold. Preferably, the sample monitored is a sample of cells which normally undergo insufficient apoptosis (*i.e.*, cancer cells).

5 By "proliferative disease" is meant a disease which is caused by or results in inappropriately high levels of cell division, inappropriately low levels of apoptosis, or both. For example, cancers such as lymphoma, leukemia, melanoma, ovarian cancer, breast cancer, pancreatic cancer, and lung cancer are all examples of proliferative disease. A neoplasm (*i.e.*, any abnormal proliferation of cells, malignant or benign), is also a proliferative disease of the
10 invention.

By a "cell proliferating in a proliferative disease" is meant a cell whose abnormal proliferation contributes to the disease. Preferably, the cell expresses the antigen PCNA.

By "polypeptide" is meant any chain of more than two amino acids, regardless of post-translational modification such as glycosylation or phosphorylation.

15 By "IAP or NAIP biological activity" is meant any activity known to be caused *in vivo* or *in vitro* by a NAIP or an IAP polypeptide. Preferred biological activities of IAP and NAIP polypeptides are those described herein, and include, without limitation, a level of expression of the polypeptide that is normal for that cell type, a level of expression of the mRNA that is normal for that cell type, an ability to block apoptosis, and an ability to be
20 cleaved.

By a "compound that decreases the biological activity" is meant a compound that decreases any activity known to be caused *in vivo* or *in vitro* by a NAIP polypeptide or an IAP polypeptide. Preferred compounds include, without limitation, an antisense nucleic acid molecule that is complementary to the coding strand of nucleic acid molecule that encodes an
25 IAP or a NAIP polypeptide; an antibody, such as a neutralizing antibody, that specifically binds to an IAP or a NAIP polypeptide; and a negative regulator of an IAP or a NAIP polypeptide, such as a polypeptide fragment that includes the ring zing finger of an IAP polypeptide, a polypeptide fragment that has no more than two BIR domains, or nucleic acid molecules encoding these polypeptide fragments.

30 By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a

- 11 -

reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software program matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "substantially pure polypeptide" is meant a polypeptide that has been separated from the components that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the polypeptide is an IAP polypeptide that is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure IAP polypeptide may be obtained, for example, by extraction from a natural source (e.g. a fibroblast, neuronal cell, or lymphocyte) by expression of a recombinant nucleic acid encoding an IAP polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components.

Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

- 12 -

By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the
5 genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (*e.g.*, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has
10 been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) an IAP polypeptide.

By "transgene" is meant any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the
15 transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

By "transgenic" is meant any cell which includes a DNA sequence which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic mammalian (*e.g.*,
20 rodents such as rats or mice) and the DNA (transgene) is inserted by artifice into the nuclear genome.

By "transformation" is meant any method for introducing foreign molecules into a cell. Lipofection, calcium phosphate precipitation, retroviral delivery, electroporation, and biolistic transformation are just a few of the teachings which may be used. For example,
25 biolistic transformation is a method for introducing foreign molecules into a cell using velocity driven microprojectiles such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts which include, but are not limited to, helium-driven, air-driven, and gunpowder-driven techniques. Biolistic transformation may be applied to the transformation or transfection of a wide variety of cell types and intact tissues including,
30 without limitation, intracellular organelles (*e.g.*, and mitochondria and chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells.

- 13 -

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (*i.e.*, facilitates the production of, *e.g.*, an IAP polypeptide, a recombinant protein or a RNA molecule).

By "reporter gene" is meant a gene whose expression may be assayed; such genes include, without limitation, glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and β -galactosidase.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the native gene.

By "operably linked" is meant that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (*e.g.*, transcriptional activator proteins are bound to the regulatory sequences).

By "conserved region" is meant any stretch of six or more contiguous amino acids exhibiting at least 30%, preferably 50%, and most preferably 70% amino acid sequence identity between two or more of the IAP family members, (*e.g.*, between human HIAP-1, HIAP-2, and XIAP). Examples of preferred conserved regions are shown (as boxed or designated sequences) in Figures 5-7 and Tables 1 and 2, and include, without limitation, BIR domains and ring zinc finger domains.

By "detectably-labelled" is meant any means for marking and identifying the presence of a molecule, *e.g.*, an oligonucleotide probe or primer, a gene or fragment thereof, or a cDNA molecule. Methods for detectably-labelling a molecule are well known in the art and include, without limitation, radioactive labelling (*e.g.*, with an isotope such as ^{32}P or ^{35}S) and nonradioactive labelling (*e.g.*, chemiluminescent labelling, *e.g.*, fluorescein labelling).

By "antisense," as used herein in reference to nucleic acids, is meant a nucleic acid sequence, regardless of length, that is complementary to a region on the coding strand of nucleic acid molecule (*e.g.*, genomic DNA, cDNA, or mRNA) that encodes an IAP or a NAIP polypeptide. The region of the nucleic acid molecule encoding an IAP or a NAIP polypeptide that the antisense molecule is complementary to may be a region within the coding region, a region upstream of the coding region, a region downstream of the coding

- 14 -

region, or a region within an intron, where the nucleic acid molecule is genomic DNA.

Preferably, the antisense nucleic acid is capable of enhancing apoptosis when present in a cell which normally does not undergo sufficient apoptosis and/or is between 8 and 25 nucleotides in length. Preferably, the increase is at least 10%, relative to a control, more preferably 25%, and most preferably 1-fold or more. It will be understood that antisense nucleic acid molecules may have chemical modifications known in the art of antisense design to enhance antisense compound efficiency.

By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally occurring organic molecules with which it is naturally associated.

10 Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., an IAP specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody that recognizes and binds a protein but
15 that does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, that naturally includes protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

20 Fig. 1 is the human XIAP cDNA sequence (SEQ ID NO: 3) and the XIAP polypeptide sequence (SEQ ID NO: 4).

Fig. 2 is the human HIAP-1 cDNA sequence (SEQ ID NO: 5) and the HIAP-1 polypeptide sequence (SEQ ID NO: 6).

Fig. 3 is the human HIAP-2 cDNA sequence (SEQ ID NO: 7) and the HIAP-2
25 polypeptide sequence (SEQ ID NO: 8).

Fig. 4 is the murine XIAP (also referred to as "MIAP-3" or "m-XIAP") cDNA sequence (SEQ ID NO: 9) and encoded murine XIAP polypeptide sequence (SEQ ID NO: 10).

- 15 -

Fig. 5 is the murine HIAP-1 (also referred to as "MIAP-1" or "m-HIAP-1") cDNA sequence (SEQ ID NO: 11) and the encoded murine HIAP-1 polypeptide sequence (SEQ ID NO: 12).

Fig. 6 is the murine HIAP-2 (also referred to as "MIAP-2" or "m-HIAP-2") cDNA sequence (SEQ ID NO: 13) and the encoded murine HIAP-2 polypeptide (SEQ ID NO: 14).

Fig. 7 is a photograph of a Northern blot illustrating human HIAP-1 and HIAP-2 mRNA expression in human tissues.

Fig. 8 is a photograph of a Northern blot illustrating human HIAP-2 mRNA expression in human tissues.

10 Fig. 9 is a photograph of a Northern blot illustrating human XIAP mRNA expression in human tissues.

Figs. 10A - 10D are graphs depicting suppression of apoptosis by XIAP, HIAP-1, HIAP-2, BCL-2, SMN, and 6-MYC.

Fig. 11 is a photograph of an agarose gel containing cDNA fragments that were 15 amplified, with HIAP 1-specific primers, from RNA obtained from Raji, Ramos, EB-3, Burkitt's lymphoma cells, and Jiyoye cells, and cells from normal placenta.

Fig. 12 is a photograph of a Western blot containing protein extracted from Jurkat and astrocytoma cells stained with an anti-XIAP antibody. The position and size of a series of marker proteins is indicated.

20 Fig. 13 is a photograph of a Western blot containing protein extracted from Jurkat cells following treatment as described in Example XII. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, anti-Fas antibody; lane 3, anti-Fas antibody and cycloheximide; lane 4, TNF- α ; lane 5, TNF- α and cycloheximide.

Fig. 14 is a photograph of a Western blot containing protein extracted from HeLa 25 cells following exposure to anti-Fas antibodies. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, cycloheximide; lane 3, anti-Fas antibody; lane 4, anti-Fas antibody and cycloheximide; lane 5, TNF- α ; lane 6, TNF- α and cycloheximide.

Figs. 15A and 15B are photographs of Western blots stained with rabbit polyclonal 30 anti-XIAP antibody. Protein was extracted from HeLa cells (Fig. 15A) and Jurkat cells (Fig. 15B) immediately, 1, 2, 3, 5, 10, and 22 hours after exposure to anti-Fas antibody.

- 16 -

Figs. 16A and 16B are photographs of Western blots stained with an anti-CPP32 antibody (Fig. 16A) or a rabbit polyclonal anti-XIAP antibody (Fig. 16B). Protein was extracted from Jurkat cells immediately, 3 hours, or 7 hours after exposure to an anti-Fas antibody. In addition to total protein, cytoplasmic and nuclear extracts are shown.

5 Fig. 17 is a photograph of a polyacrylamide gel following electrophoresis of the products of an *in vitro* XIAP cleavage assay.

Figs. 18 and 19 shows the increased level of HIAP-1 and HIAP-2 mRNA, respectively, in breast cancer cell lines having p53 mutations (lanes 5-7). The bottom portion of the figure shows the control.

10 Fig. 20 shows the influence of Taxol on DNA fragmentation in Cisplatin-sensitive (right) and resistant (left) human ovarian epithelial cancer cells.

Fig. 21 shows the influence of Cisplatin on DNA fragmentation in sensitive (right) and resistant (left) human ovarian epithelial cancer cells.

15 Fig. 22 shows the effects of Taxol on XIAP and HIAP-2 protein levels in Cisplatin sensitive (right) and resistant (left) human ovarian epithelial cancer cells.

Figs. 23A and 23B show the influence of Taxol and TGF β on HIAP-2 mRNA levels in Cisplatin sensitive (right) and resistant (left) human epithelial cancer cells.

Figs. 24A and 24B show the effect of TGF β on XIAP protein expression (Fig. 24A) and DNA fragmentation (Fig. 24B) in Cisplatin-sensitive (OV2008) and cisplatin-resistant
20 (C13) cells.

Fig. 25 is a series of bar graphs showing the effect of XIAP and HIAP-2 down-regulation on ovarian epithelial cancer cell viability and number. The top two panels show dead cells as a percentage of total cell population. The bottom two panels illustrate total cell number at the end of the infection period. Data represents the mean \pm SEM of four
25 experiments. ** p <0.01, *** p <0.001 (compared to vector control).

Fig. 26A is a set of photographs showing the influence of XIAP down-regulation on whole cell morphology (phase contrast; black arrows indicate cell detachment) in OV2008 cells after 60 hours of adenovirus infection with vector only (left) or adenoviral antisense XIAP (right). MOI=5 (1X; "a" and "b"); magnification 400X.

30 Fig. 26B is a series of photographs ("a" through "d") showing the influence of XIAP down-regulation on nuclear morphology (Hoechst staining; white arrows show nuclear

- 17 -

fragmentation) in OV2008 cells after 60 hours of adenovirus infection with vector only ("a" and "c") or adenoviral antisense XIAP ("b" and "d"). MOI=5 (1X; "a" and "b") and MOI=10 (2X; "c" and "d"); magnification 400X.

Fig. 26C is a bar graph showing the influence of XIAP down-regulation on the extent of apoptosis in OV2008 cells after 60 hours of no treatment, adenovirus infection with vector only, or adenovirus infection with antisense XIAP. Data represents the mean \pm SEM of three to four experiments. MOI=5 (1X) and MOI=10 (2X); * p <0.05, ** p <0.01 (compared to vector control).

Fig. 26D is a representative Western blotting analysis showing effective XIAP antisense infection in OV2008 cells after 60 hours of no treatment, adenovirus infection with vector only, or adenovirus infection with antisense XIAP. Lanes are, from left to right: control, vector (1X), vector (2X), antisense XIAP (1X), and antisense XIAP (2X). MOI=5 (1X) and MOI=10 (2X).

Fig. 26E is a bar graph showing changes in XIAP protein content in OV2008 cells after 60 hours of no treatment, adenovirus infection with vector only, or adenovirus infection with antisense XIAP, as analyzed densitometrically, using a Molecular Dynamic Phosphorimager. Data represents the mean \pm SEM of three to four experiments. MOI=5 (1X) and MOI=10 (2X); * p <0.05, ** p <0.01 (compared to vector control).

Fig. 27A is a series of photographs showing effects of cisplatin-induced apoptosis (at 0 and 30 μ M cisplatin in a 24 hour culture) the nuclear morphology of cisplatin-sensitive cells (OV2008; left two photographs) and cisplatin-resistant cells (C13; right two photographs), using Hoechst staining, magnification 400X; arrows show fragmented nuclei.

Fig. 27B is a set of photographs showing agarose gel immobilized electrophoretically resolved apoptotic low molecular weight DNA fragmentation from cisplatin treated OV2008 and C13 cells.

Fig. 27C is a line graph showing a concentration-response study of apoptosis in OV2008 and C13 cells following 24 hours of culture in 0, 10, 20, and 30 μ M cisplatin. Data represents the mean \pm SEM of three experiments. ** p <0.01 (compared to control).

Fig. 28A is a series of representative Western blotting analyses showing concentration-dependent inhibition of XIAP and HIAP-2 protein expression in cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) ovarian epithelial cancer cells following 24

- 18 -

hour culture with 0, 10, 20, and 30 μ M cisplatin. Equal amounts of solubilized proteins (20-60 μ g/lane, depending on the individual experiment) were analyzed by Western blot using anti-human XIAP or anti-HIAP-2 antibodies.

Fig. 28B is a panel of bar graphs showing the changes in XIAP (left two graphs) and HIAP-2 (right two graphs) protein content as analyzed densitometrically, using the Molecular Dynamics Phosphoimager, for cisplatin-treated (24 hours at indicated concentration) OV2008 cells (upper two graphs) and C13 cells (lower two graphs). Data represents the mean \pm SEM of three experiments. * p <0.05, ** p <0.01 (compared to control).

Fig. 29A is a series of representative Western blotting analyses showing concentration-dependent inhibition of XIAP and HIAP-2 protein expression in cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) ovarian epithelial cancer cells following 6, 12, or 24 hours of culture with or without 30 μ M cisplatin. Equal amounts of solubilized proteins (20-60 μ g/lane, depending on the individual experiment) were analyzed by Western blot using anti-human XIAP or anti-HIAP-2 antibodies.

Fig. 29B is a panel of bar graphs showing the changes in XIAP (left two graphs) and HIAP-2 (right two graphs) protein content as analyzed densitometrically, using the Molecular Dynamics Phosphoimager, for OV2008 cells (white bars) and C13 cells (black bars) cultured with or without 30 μ M cisplatin for 6, 12, or 24 hours. Data represents the mean \pm SEM of three experiments. * p <0.05, ** p <0.01 (compared to control).

Fig. 30A is a series of representative Western blotting analyses showing concentration-dependent inhibition of XIAP and HIAP-2 protein expression in cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) ovarian epithelial cancer cells following hours of culture with or without 30 μ M cisplatin. Equal amounts of solubilized proteins (40-60 μ g/lane, depending on the individual experiment) were analyzed by Western blot using anti-human XIAP or anti-HIAP-2 antibodies.

Fig. 30B is a panel of bar graphs showing the changes in XIAP (top graph) and HIAP-2 (bottom graph) protein content as analyzed densitometrically, using the Molecular Dynamics Phosphoimager, for A2780s cells (left) and A2780cp cells (right) cultured with (black bars) or without (white bars) 30 μ M cisplatin for 24 hours. Data represents the mean \pm SEM of three experiments. ** p <0.01 (compared to control).

- 19 -

Fig. 31A is set of photographs ("a" through "d") showing the effects of XIAP overexpression on the apoptotic action of cisplatin (30 μ M) on nuclear morphology of cisplatin-sensitive OV2008 cells after 48 hours of infection of these cells with adenoviral sense XIAP cDNA or vector only (control). At a magnification of 400X. "a", vector (no cisplatin); "b", sense XIAP (no cisplatin); "c", vector plus cisplatin-treatment; "d", sense XIAP plus cisplatin treatment.

Fig. 31B is a graph showing the percentage of total cell population undergoing apoptosis of 30 μ M cisplatin-treated OV2008 cells following 48 hours of infection of these cells with adenoviral sense XIAP cDNA or vector only (control). Data represent mean \pm SEM of three experiments. * p <0.05, *** p <0.001 (compared to vector control); \bar{p} <0.01, $\bar{\bar{p}}$ <0.001 (compared to vector plus cisplatin group).

Fig. 31C is a representative Western blotting analysis showing changes in XIAP protein content in OV2008 cells following infection with adenoviral sense XIAP cDNA or vector only (control) with or without treatment with 30 μ M cisplatin. Lanes are, from left to right: control, vector, vector plus cisplatin, sense XIAP, and sense XIAP plus cisplatin.

Fig. 31D is a graph showing the changes in XIAP protein content in OV2008 cells following infection with adenoviral sense XIAP cDNA or vector only (control) with or without treatment with 30 μ M cisplatin, as analyzed densitometrically, using the Molecular Dynamic Phosphoimager. Data represent mean \pm SEM of three experiments. * p <0.05, *** p <0.001 (compared to vector control); \bar{p} <0.01, $\bar{\bar{p}}$ <0.001 (compared to vector + cisplatin group).

Figs. 32A-32D are a series of photographs showing the *in situ* detection of apoptosis (using TUNEL) and immunolocalization of PCNA, XIAP and HIAP-2 in human ovarian surface epithelial tumour tissue. Fig. 32A indicates the *in situ* TUNEL localization of apoptotic cells. Figs. 32B, 32C, and 32D represent immuno-reactivates for PCNA, XIAP and HIAP-2, respectively. The regions of tumor shown in the circle and the rectangle in each of Figs. 32A-32D was TUNEL-positive and TUNEL-negative, respectively. Magnification is 400X.

Detailed Description

- 20 -

Previously, we have provided a novel family of inhibitors of apoptosis, the IAPs, and an additional related anti-apoptotic protein, NAIP. Here we provide identification of cancer types in which dysregulation of the IAPs and NAIP is apparent. Our results are of paramount importance and provide diagnostics, prognostics, treatments, and drug screens aimed at the
5 detection and effective treatment of cancer.

Cancer Screening

We initially studied IAP and NAIP expression levels in a variety of normal tissues and cancer cell lines using commercially available northern blots. Elevated XIAP, HIAP-1 and HIAP-2 mRNA was noted in a surprising number of cancer lines of diverse lineage,
10 including colorectal cancer, lymphoma, leukemia, and melanoma cell lines. In contrast, BCL-2 mRNA was elevated in only a single cell line. Although this result reinforced the importance of the IAPs and NAIP in cancer, the question remained as to whether the individual cancer cell lines on the blot were representative of the cancer type. As a result, we screened panels of cancer cell lines of particular tumor type by northern blot and quantitative
15 RT-PCR analysis in order to ascertain the frequency of IAP and NAIP dysregulation. The results are summarized as follows:

Burkitt's Lymphoma.

We studied both the frequency and consequences of IAP upregulation in Burkitt's lymphoma. Elevated levels of HIAP-1 and HIAP-2 have been found in the vast majority of
20 the Burkitt's cell lines examined. Furthermore, those Burkitt's lines expressing low levels of HIAP-1 are transcriptionally activated by Epstein-Barr virus (EBV) infection.

Breast Adenocarcinoma.

A key observation was made in this survey, in which a correlation was observed between drug resistance, p53 status, and HIAP-1 and HIAP-2 expression. Four of the cell
25 lines possessed wild-type p53, while three possessed documented p53 mutations that correlated with resistance to the anti-cancer drug adriamycin. Significantly, the three lines which were relatively more drug resistant also displayed elevated HIAP-1 and HIAP-2

- 21 -

mRNA levels. These results indicate that one of the ways that p53 controls apoptosis is through regulation of these genes.

Ovarian Carcinoma.

mRNA *in situ* analysis suggest a role for NAIP in the developmental biology of the
5 ovary. Overexpression of HIAP-2 and XIAP mRNA has also been documented in some ovarian cancer cell lines.

Pancreatic Cancer.

Approximately 25% of the pancreatic cancer cell lines tested to date demonstrate HIAP-1 and HIAP-2 mRNA elevation.

10 *Summary of Cancer Panels.*

To date, a significant fraction of cancer cell lines of each type examined display elevated IAP levels. Increased NAIP levels are also implicated in cancer. Our results indicate that HIAP-1 and HIAP-2 tend to be the most frequently and dramatically upregulated. The apparent coordinate regulation of both genes was surprising given that the
15 normal tissue distribution of these proteins is very different. Our observations are strengthened by the fact that HIAP-1 and HIAP-2 reside in tandem array on chromosome 11q23, a site frequently rearranged in lymphomas and leukemias.

Transcriptional regulation of the IAPs in cancer cell lines.

Our experiments have established a correlation between p53 status and transcriptional
20 overexpression of HIAP-1 and HIAP-2. This provides an important new way in which to enhance apoptosis, particularly in view of the fact that the mechanism by which p53 controls cell fate remains largely unknown. It has previously been documented that wild-type p53 negatively down-regulates BCL-2, and positively upregulates the BCL-2 antagonist BAX. In some cancer cell types, mutation of p53 causes a two-fold effect; namely, the upregulation of
25 BCL-2, and down regulation of BAX, both of which contribute to the anti-apoptotic phenotype. While not wishing to bind ourselves to a particular theory, we believe that wild-type p53 also transcriptionally suppresses HIAP-1 and HIAP-2. DNA damage that includes

- 22 -

the increase in wild-type levels p53 levels would therefore result in decreased HIAP-1 and HIAP-2 in normal cells, resulting in apoptosis. Mutations in the p53 gene would therefore result in a loss of transcriptional control of these IAP genes. As a result, p53 mutant cancer cells would display constitutively high levels of HIAP-1 and HIAP-2, rendering the cells
5 resistant to anti-cancer therapies. The p53/HIAP-1 and HIAP-2 correlations may be extended to the other cancer cell line panels. One may directly demonstrate p53 regulation of the IAPs using transfection assays and northern blot analysis.

Accordingly, we predict that cancer cells having p53 mutations (p53*) will have increased IAP levels resulting in a poor response to chemotherapeutics. Because IAP levels
10 may be assessed more readily than the presence of a p53* mutation, our discovery also provides an important improvement in cancer diagnosis and prognosis (see below).

Transgenic Mice

We have constructed a number of IAP and NAIP transgenic mouse expression vectors, including T-cell, B-cell, and neuronal specific promoter constructs. Founder mice
15 have been identified and are viable, and, for most of these constructs, we have developed breeding colonies. These mice will likely be prone to cancers of the tissue types in which the promoter is active. Thus the mice provide an excellent resource for testing the efficacy of anti-sense oligonucleotides and for screening for apoptosis-enhancing cancer therapeutics. Standard mouse drug screening models and gene delivery protocols may be employed to
20 utilize the mice for this purpose.

Diagnostic/Prognostic Reagents

There is a relative lack of diagnostic and prognostic tests which clinical oncologists may utilize in determining the appropriate degree of intervention in the treatment of cancer. Mutation of the p53 gene remains one of the best prognostic indicators in cancer biology.
25 However, the number of different mutations identified to date is great and the mutations are scattered throughout the gene. In addition, many mutations in p53 result in an inappropriate stabilization of the protein, which allows detection at the protein level rather than at the mRNA level. Mutations which alter the transactivation/repression activities of the protein are not necessarily apparent at either the mRNA or protein levels. On the other hand, if IAP

- 23 -

and NAIP expression levels correlate with p53 mutation they may provide more valuable prognostic information and assist in the determination of which patients require more aggressive treatment or which patients are, perhaps, not treatable with currently approved therapies. This latter class of patients may be identified as ideal candidates for clinical testing of new cancer therapeutics, particularly those which decrease IAP levels or act in a manner independent of the anti-apoptotic pathway.

Thus, the invention provides at least two assays for prognosis and diagnosis. Semi-quantitative RT-PCR based assays may be used to assay for IAP and/or NAIP gene or protein expression levels. Alternatively, monoclonal antibodies may be incorporated into an ELISA (enzyme-linked immunosorbent assay) -type assay for direct determination of protein levels.

Therapeutic Products

For IAP or NAIP-related therapies, one may employ the paradigms utilized for BCL-2 and RAS antisense development, although, in contrast to RAS antisense, accommodation of mutations is not required. Most useful are antisense constructs which enhance apoptosis at least 10%, preferably by enhancing degradation of the RNA in the nucleus.

In addition to the antisense approaches described herein, the invention features small molecule screening assays which may be used to identify lead compounds that negatively regulate the IAPs or NAIP. For example, compounds which enhance apoptosis in the presence of IAP overexpression or which decrease the level of IAP biological activity may be detected and are useful cancer therapeutics.

Molecules that are found, by the methods described herein, to effectively modulate IAP gene expression or polypeptide activity may be tested further in standard animal cancer models. If they continue to function successfully in an *in vivo* setting, they may be used as therapeutics to either inhibit or enhance apoptosis, as appropriate.

25 *Manipulation of cancer chemotherapeutic drug resistance using an antisense oligonucleotide and fragment approaches.*

We have documented that overexpression of the IAPs renders cell lines resistant to serum growth factor withdrawal, tumor necrosis factor alpha (TNF) and menadione exposure, all of which are treatments that normally induce apoptosis. Herein we describe the extension

- 24 -

of these studies to cancer cell lines using apoptotic triggers used in clinical situations, such as doxorubicin, adriamycin, and methotrexate. Our findings have led up to design antisense RNA therapeutics. Rapid screening of multiple cell lines for apoptotic response has been made feasible through the generation of a series of sense and antisense adenoviral IAP and
5 NAIP expression vectors, as well as control lacZ viruses. One may now show enhanced drug resistance or enhanced drug sensitivity using these expression constructs. In addition, antisense adenovirus constructs have been developed and used to test reversal of the drug resistant phenotype of appropriate cell lines.

We have surveyed cancer cell lines with the objective of identifying tumor types in
10 which IAP or NAIP overexpression is apparent or altered and these results are described both above and in the Examples below. Concomitant to this research, we have designed a series of antisense oligonucleotides to various regions of each of the IAPs. After testing in an assay system, *i.e.*, with the adenoviral vectors system, these oligonucleotides, as well as antisense oligonucleotides to various regions of NAIP, may be used to enhance drug
15 sensitivity. Animal modeling of the effectiveness of antisense IAP and NAIP oligonucleotides may also be employed as a step in testing and appropriate transgenic mammals for this are described above and also generally available in the art.

The following describes some of the testing systems which may be employed.

Anti-Cancer Gene Therapy

20 Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells requiring enhanced apoptosis (for example, breast cancer and ovarian cancer cells) may be used as an oligonucleotide transfer delivery system for a therapeutic constructs.

Alternatively, standard non-viral delivery methods may be used. Numerous vectors
25 useful for viral delivery are generally known (Miller, A.D., Human Gene Therapy 1: 5-14, 1990; Friedman, T., Science 244: 1275-1281, 1989; Eglitis and Anderson, BioTechniques 6: 608-614, 1988; Tolstoshev and Anderson, Curr. Opin. Biotech. 1: 55-61, 1990; Cornetta *et al.*, Prog. Nucl. Acid Res. and Mol. Biol. 36: 311-322, 1987; Anderson, W. F., Science 226: 401-409, 1984; Moen, R. C., Blood Cells 17: 407-416, 1991; Miller *et al.*, BioTechniques 7:

- 25 -

980-990, 1989; Le Gal La Salle *et al.*, Science 259: 988-990, 1993; and Johnson, Chest 107: 77S-83S, 1995).

Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg *et al.*, New Engl. J. Med. 323: 570-578, 1990; Anderson *et al.*, U.S.

5 Patent No. 5,399,346).

Non-viral approaches may also be employed for the introduction of therapeutic nucleic acid molecules (*e.g.*, oligonucleotides) into cells otherwise predicted to undergo apoptosis. For example, IAP may be introduced into a neuron or a T cell by lipofection (Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84: 7413-7417, 1987; Ono *et al.*, Neurosci. Lett. 10 117: 259-263, 1990; Brigham *et al.*, Am. J. Med. Sci. 298: 278-281, 1989; Staubinger *et al.*, Meth. Enz. 101: 512-527, 1983), asialorosanucoid-polylysine conjugation (Wu *et al.*, J. Biol. Chem. 263: 14621-14624, 1988; Wu *et al.*, J. Biol. Chem. 264: 16985-16987, 1989); direct deliver in saline; or, less preferably, microinjection under surgical conditions (Wolff *et al.*, Science 247: 1465-1468, 1990).

15 For any of the methods of application described above, the therapeutic nucleic acid construct is preferably applied to the site of the needed apoptosis event (for example, by injection). However, it may also be applied to tissue in the vicinity of the predicted apoptosis event, to a blood vessel supplying the cells predicted to require enhanced apoptosis, or orally.

In the constructs described, nucleic acid expression can be directed from any suitable 20 promoter (*e.g.*, the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in ovarian cells, breast tissue, neural cells, T cells, or B cells may be used to direct expression. The enhancers used could include, without limitation, those that are characterized as tissue- 25 or cell-specific in their expression. Alternatively, if a clone used as a therapeutic construct, regulation may be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

Anti-cancer therapy is also accomplished by direct administration of the therapeutic 30 sense IAP nucleic acid or antisense IAP nucleic acid (*e.g.*, oligonucleotides) to a cell that is expected to require enhanced apoptosis. The nucleic acid molecule may be produced and

- 26 -

isolated by any standard technique, but is most readily produced by *in vitro* transcription using an IAP related nucleic acid under the control of a high efficiency promoter (*e.g.*, the T7 promoter), or, by organic synthesis techniques (for, *e.g.*, oligonucleotides).

Administration of IAP antisense nucleic acid to malignant cells can be carried out by any of
5 the methods for direct nucleic acid administration described above, or any method otherwise known in the art.

Another therapeutic approach within the invention involves administration of recombinant IAP protein fragments or IAP antibodies, either directly to the site where enhanced apoptosis is desirable (for example, by injection) or systemically (for example, by
10 any conventional recombinant protein administration technique).

The dosage of a NAIP or an IAP protein, a polypeptide fragment thereof, a mutant thereof, or antibodies that specifically bind NAIP or an IAP polypeptide depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 500 mg inclusive are administered per day to an adult in any
15 pharmaceutically acceptable formulation.

Administration of IAP and NAIP Polypeptides, Nucleic Acids, and Inhibitors of IAP or NAIP Synthesis or Function

An IAP or NAIP mutant protein or protein fragment, a nucleic acid molecule encoding the same, a nucleic acid molecule encoding an IAP or NAIP antisense nucleic acid,
20 or a inhibitor of an IAPs or NAIP may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to patients suffering from a disease that is caused by excessive cell proliferation.

Administration may begin before the patient is symptomatic.

25 Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intrathecal, intracapsular, intracisternal, intraperitoneal, intranasal, aerosol, suppository, or oral administration. For example, therapeutic formulations may be in the form of liquid solutions or suspensions; for oral

- 27 -

administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences, (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for IAP or NAIP modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

If desired, treatment with an IAP or NAIP mutant proteins or IAP or NAIP fragments, related genes, or other modulatory compounds may be combined with more traditional therapies for the proliferative disease such as surgery or chemotherapy.

Detection of Conditions Involving Insufficient Apoptosis

IAP and NAIP polypeptides and nucleic acid sequences find diagnostic use in the detection or monitoring of conditions involving insufficient levels of apoptosis, *i.e.*, proliferative disease. For example, increased expression of IAPs or NAIP, alterations in localization, and IAP or NAIP cleavage correlate with inhibition of apoptosis and cancer in humans. Accordingly, an increase in the level of IAP or NAIP production may provide an indication of a proliferative condition or a predisposition to such a condition. Levels of IAP or NAIP expression may be assayed by any standard technique. For example, IAP or NAIP expression in a biological sample (*e.g.*, a biopsy sample) may be monitored by standard Northern blot analysis or may be aided by PCR (see, *e.g.*, Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1994; PCR Technology: Principles and

- 28 -

Applications for DNA Amplification, H.A. Ehrlich, Ed., Stockton Press, NY: Yap *et al.*, Nucl. Acids. Res. 19: 4294, 1991).

Alternatively, a biological sample obtained from a patient may be analyzed for one or more mutations in the IAP or NAIP sequences or p53 sequences using a mismatch detection
5 approach. Generally, these techniques involve PCR amplification of nucleic acid from the patient sample, followed by identification of the mutation (*i.e.*, mismatch) by either altered hybridization, aberrant electrophoretic gel migration, binding or cleavage mediated by mismatch binding proteins, or direct nucleic acid sequencing. Any of these techniques may be used to facilitate mutant IAP or NAIP detection, and each is well known in the art;
10 examples of particular techniques are described, without limitation, in Orita *et al.*, Proc. Natl. Acad. Sci. USA 86: 2766-2770, 1989; Sheffield *et al.*, Proc. Natl. Acad. Sci. USA 86: 232-236, 1989).

In yet another approach, immunoassays are used to detect or monitor IAP or NAIP protein in a biological sample. IAP or NAIP-specific polyclonal or monoclonal antibodies
15 (produced as described above) may be used in any standard immunoassay format (*e.g.*, ELISA, Western blot, or RIA) to measure IAP or NAIP polypeptide levels from cancerous control cells. These levels would be compared to wild-type IAP or NAIP levels, with a decrease in IAP production relative to a wild-type cell indicating a condition involving increased apoptosis and a decrease relative to a known cancer cell indicating a decreased
20 likelihood of an IAP or NAIP-related cancer. Examples of immunoassays are described, *e.g.*, in Ausubel *et al.*, *supra*. Immunohistochemical techniques may also be utilized for IAP or NAIP detection. For example, a tissue sample may be obtained from a patient, sectioned, and stained for the presence of IAP or NAIP using an anti-IAP or anti-NAIP antibodies and any standard detection system (*e.g.*, one which includes a secondary antibody conjugated to
25 horseradish peroxidase). General guidance regarding such techniques can be found in, *e.g.*, Bancroft and Stevens (Theory and Practice of Histological Techniques, Churchill Livingstone, 1982) and Ausubel *et al.* (*supra*).

In one preferred example, a combined diagnostic method may be employed that begins with an evaluation of IAP or NAIP protein production (for example, by
30 immunological techniques or the protein truncation test (Hogerrorst *et al.*, Nature Genetics 10:208-212, 1995)) and also includes a nucleic acid-based detection technique designed to

- 29 -

identify more subtle IAP or NAIP alterations, e.g., mutations. As described above, a number of mismatch detection assays are available to those skilled in the art, and any preferred technique may be used. Mutations in IAP or NAIP may be detected that either result in enhanced IAP or NAIP expression or alterations in IAP or NAIP biological activity. In a variation of this combined diagnostic method, IAP or NAIP biological activity is measured as anti-apoptotic activity using any appropriate apoptosis assay system (for example, those described above).

Mismatch detection assays also provide an opportunity to diagnose an IAP-mediated or an NAIP-mediated predisposition to diseases caused by insufficient apoptosis. For example, a patient heterozygous for an IAP or a NAIP mutation may show no clinical symptoms and yet possess a higher than normal probability of developing one or more types of proliferative diseases. Given this diagnosis, a patient may take precautions to minimize their exposure to adverse environmental factors (for example, UV exposure or chemical mutagens) and to carefully monitor their medical condition (for example, through frequent physical examinations). This type of IAP or NAIP diagnostic approach may also be used to detect IAP or NAIP mutations in prenatal screens. The IAP or NAIP diagnostic assays described above may be carried out using any biological sample (for example, any biopsy sample or bodily fluid or tissue) in which IAP or NAIP is normally expressed. Identification of a mutant IAP or NAIP gene may also be assayed using these sources for test samples.

Alternatively, an alteration in IAP or NAIP activity, particularly as part of a diagnosis for predisposition to IAP-associated or NAIP-associated proliferative disease, may be tested using a nucleic acid sample from any cell, for example, by mismatch detection techniques. Preferably, the DNA sample is subjected to PCR amplification prior to analysis.

The following examples are meant to illustrate, not limit, the invention.

EXAMPLE 1: ELEVATED IAP LEVELS IN CANCER CELL LINES

In order to specifically demonstrate the utility of IAP gene sequences as diagnostics and prognostics for cancer, a Human Cancer Cell Line Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7757-1) was probed. This Northern blot contained approximately 2 µg of poly A⁺ RNA per lane from eight different human cell lines: (1) promyelocytic leukemia HL-60, (2) HeLa cell S3, (3) chronic myelogenous leukemia K-562, (4)

- 30 -

lymphoblastic leukemia MOLT-4, (5) Burkitt's lymphoma Raji, (6) colorectal adenocarcinoma SW480, (7) lung carcinoma A549, and (8) melanoma G361. As a control, a Human Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7759-1) was probed. This Northern blot contained approximately 2 µg of poly A⁺ RNA from eight different human tissues: (1) spleen, (2) thymus, (3) prostate, (4) testis, (5) ovary, (6) small intestine, (7) colon, and (8) peripheral blood leukocytes.

The Northern blots were hybridized sequentially with: (1) a 1.6 kb probe to the XIAP coding region, (2) a 375 bp HIAP-2 specific probe corresponding to the 3' untranslated region, (3) a 1.3 kb probe to the coding region of HIAP-1, which cross-reacts with HIAP-2, (4) a 1.0 kb probe derived from the coding region of BCL-2, and (5) a probe to β-actin, which was provided by the manufacturer. Hybridization was carried out at 50°C overnight, according to the manufacturer's suggestion. The blot was washed twice with 2X SSC, 0.1% SDS at room temperature for 15 minutes and then with 2X SSC, 0.1% SDS at 50°C.

All cancer lines tested showed increased IAP expression relative to samples from non-cancerous control tissues (Table 1). Expression of XIAP was particularly high in HeLa (S-3), chronic myelogenous leukemia (K-562), colorectal adenocarcinoma (SW-480), and melanoma (G-361) lines. Expression of HIAP-1 was extremely high in Burkitt's lymphoma, and was also elevated in colorectal adenocarcinoma. Expression of HIAP-2 was particularly high in chronic myelogenous leukemia (K-562) and colorectal adenocarcinoma (SW-480). Expression of BCL-2 was upregulated only in HL-60 leukemia cells.

TABLE 1

- 31 -

NORTHERN BLOT IAP RNA LEVELS IN CANCER CELLS*

	XIAP	HIAP-1	HIAP- 2
Promyelocytic Leukemia HL-60	+	+	+
Hela S-3	+	+	+
Chronic Myelogenous Leukemia K-562	+++	+	+++
5 Lymphoblastic Leukemia MOLT-4	+++	+	+
Burkitt's Lymphoma Raji	+	+(x10)	+
Colorectal Adenocarcinoma SW-480	+++	+++	+++
Lung Carcinoma A-549	+	+	+
Melanoma G-361	+++	+	+

10 *Levels are indicated by a (+) and are the approximate increase in RNA levels relative to Northern blots of RNA from non-cancerous control cell lines. A single plus indicates an estimated increase of at least 1-fold

These observations indicate that upregulation of the anti-apoptotic IAP genes may be a widespread phenomenon in proliferative diseases, perhaps occurring much more frequently than upregulation of BCL-2. Furthermore, upregulation may be necessary for the establishment or maintenance of the transformed state of cancerous cells.

In order to pursue the observation described above, *i.e.*, that HIAP-1 is overexpressed in the Raji Burkitt's lymphoma cell line, RT-PCR analysis was performed in multiple Burkitt's lymphoma cell lines. Total RNA was extracted from cells of the Raji, Ramos, EB-3, and Jiyoye cell lines, and as a positive control, from normal placental tissue. The RNA was reverse transcribed, and amplified by PCR with the following set of oligonucleotide primers:

5'-AGTGCGGGTTTTTATTATGTG-3' (SEQ ID NO: 15) and

5'-AGATGACCACAAGGAATAAACACTA-3' (SEQ ID NO: 16), which selectively

25 amplify a hiap-1 cDNA fragment. RT-PCR was conducted using a Perkin Elmer 480 Thermocycler to carry out 35 cycles of the following program: 94°C for 1 minute, 50°C for 1.5 minutes, and 72°C for 1 minute. The PCR reaction product was electrophoresed on an agarose gel and stained with ethidium bromide. Amplified cDNA fragments of the appropriate size were clearly visible in all lanes containing Burkitt's lymphoma samples, but

- 32 -

absent in the lanes containing the normal placental tissue sample, and absent in lanes containing negative control samples, where template DNA was omitted from the reaction (Fig. 11).

EXAMPLE 2: IAPs IN BREAST CANCER

- 5 The following data relate to the regulation and role of IAPs in cancer cells. Figs. 18 and 19 show data demonstrating that HIAP-1 and HIAP-2 are both upregulated in breast cancer cell lines that contain mutant p53. The lanes contain 20 µg of total RNA from the following lines: 1. MCF-7 (clone 1, wt p53); 2. MCF-7 (clone 2, wt p53); 3. MCF-7 (American Type Culture Collection, wt p53); 4. MCF-7 (parental line, California, wt p53); 5. 10 MCF-7 (California, adriamycin resistant variant, mutant p53); 6. MDA MB 231 (ATCC, mutant p53, codon 280); 7. T47-D (ATCC, mutant p53, codon 194); 8. ZR-75 (ATCC, wt p53). The amount of RNA loaded on each gel was controlled for by hybridization with glycerol phosphate dehydrogenase (GAPDH).

EXAMPLE 3: IAPS IN OVARIAN CANCER

15 *Overview.*

Epithelial ovarian cancer is the leading cause of death from gynecologic malignancy. Although clinical and histologic prognostic factors such as tumor grade and surgical stage are well understood, the biologic process that leads to uncontrolled cellular growth is less clear. The control of cell numbers during tissue growth is thought to be the results of a balance of 20 cell proliferation and cell death. An aberration in this natural homeostasis likely contributes to malignant cellular transformation.

Recent studies on ovarian cancer cell biology have suggested that the deregulation of apoptosis may be one of the underlying pathologic mechanism in this disease. However, the molecular mechanisms involved in its regulation is poorly understood and the role and 25 regulation of the IAP genes in ovarian cell transformation have not been examined previously. Ovarian epithelial cancer is in part a result of suppressed apoptosis of ovarian surface epithelial cells. The effectiveness of certain chemotherapeutic agents rests on their ability to induce cell death. The loss of responsiveness of the cells to these agents is due to a desensitization of the apoptotic process to these agents. The regulation of ovarian epithelial

- 33 -

cell apoptosis involves changes in the expression of IAP genes and post-translational modification/processing of the IAP gene products.

We have conducted experiments and now believe that IAPs play a key role in maintaining the normal growth of ovarian surface epithelial cells and that the overexpression of these genes leads to cellular transformation. Furthermore, we have discovered that the effectiveness of chemotherapeutic agents in the treatment of this form of malignancy rests upon their ability to suppress the expression of the IAP genes. By seeking to control the regulation of the IAP genes in human ovarian epithelial cancer cells we have provided a rational approach for the development of new chemotherapeutics for patients both responsive and resistant to current cancer drugs. Similarly, assays designed to detect compounds which decrease IAP biological activity provide a rational method for drug discovery.

Methods.

a) Human Ovarian Epithelial Cancer Cell Culture

Cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) human ovarian epithelial cells were cultured in a chemically-defined medium at 37°C for up to 48 hours in the presence or absence of TGFβ (20 ng/ml), taxol (0 - 1.0 μM) or cisplatin (0 - 30 μM). At the end of the culture period, cells were either fixed for immunocytochemistry and TUNEL analyses, or snap frozen for subsequent extraction for IAP mRNA and proteins analyses.

b) Identification of Cell Death

For nuclear staining, human ovarian epithelial cancer cells were fixed (4% formalin in PBS; 10 min., room temp.), washed in PBS, resuspended in Hoechst 33248 stain (0.1 μg/ml PBS, 10 min) washed again and spotted onto slides for microscopy. Nuclear staining was observed and photographed using a Zeiss fluorescent microscope equipped with an FITC filter. Apoptotic cells were identified by typical nuclear morphology, and counted using randomly selected fields and numbered photographic slides to avoid bias during counting.

For quantitation of DNA ladders, cellular DNA was extracted using the Qiagen Blood kit (Qiagen Inc., Chatsworth, CA). DNA was quantified by ethidium bromide fluorescence. DNA (0.5 μg) was then end labelled by incubating (20 min., room temp.) with Klenow enzyme (2 U in 10 mM Tris plus 5 mM MgCl₂) and 0.1 μCi [α-³²P]dCTP. Unincorporated

- 34 -

nucleotides were removed with the Qiagen nucleotide removal kit and samples were resolved by Tris-acetate-EDTA agarose (1.8%) gel electrophoresis. The gel was then dried (2 hours, no heat) and exposed to a Bio-Rad phosphoimager screen to densitometrically quantify low molecular weight DNA (<15 kilo base-pairs), and subsequently to X-ray film at -80°C.

5 For *in situ* TUNEL labelling of apoptotic cells to identify cell death, the *in situ* cell death detection kit (Boehringer-Mannheim, Indianapolis, IN) was used, according to manufacturer's instructions. Slides prepared for histology were treated (20 min. at 37°C) with terminal transferase in the presence of FITC-conjugated dUTP.

c) Western Blot Analyses for IAPs

10 Protein extracts were prepared from human surface epithelial cancer cells sonicated (8 sec/cycle, 3 cycles) on ice in sucrose buffer (0.25 M sucrose, 0.025 M NaCl, 1 mM EGTA and 15 mM Tris-HCl pH 6.8, supplemented with 1 mM PMSF, 2 µg/ml of leupeptin and 5 µg/ml of aprotinin. The sonicates were centrifuged at 13,000xg for 10 min., the supernatants were collected and stored at -20°C until electrophoretic analyses were performed. Protein
15 concentration was determined by Bio-Rad Protein Assay. Proteins (10-30 µg) were resolved by one-dimensional SDS-PAGE, and electrophoretically transferred to nitrocellulose membrane. Membranes were blocked with 5% non-fat milk, and subsequently incubated with rabbit polyclonal antibody for IAP [anti-human HIAP-2ΔE (960529; 1:1000 dilution), anti-human NAIP E1.0 (951015; 1:1000 dilution) or anti-human XIAP (1:1000 dilution)]
20 diluted in TBST (10 mM Tris-buffered saline, 0.1% Tween-20, pH7.5) containing 5% milk. An ECL kit was used to visualize immunopositive protein (Amersham Intl., Arlington Heights, IL).

d) Northern Blots for IAP mRNAs

 Total RNA from ovarian surface epithelial cancer cells by using RNeasy Kit
25 (Qiagen). The RNA samples (10-15 µg) were quantified spectrophotometrically and size-fractionated by electrophoresis on formaldehyde-agarose gels (1.1%) containing 1 µg/ml ethidium bromide to confirm even loading of RNA samples and adequate separation of 28S and 18S ribosomal bands. The RNAs bands were blotted onto a nylon membrane and cross-linked by UV light. Membranes were prehybridized in 50% formamide, saline sodium citrate

- 35 -

(SSC; 750 mM NaCl, 75 mM sodium citrate), 1X Denhardt's solution, 1% SDS, 4 mM EDTA and 100 µg/ml sheared salmon sperm DNA for 4 hours at 42°C. Hybridization was performed overnight at 42 °C with 20 million cpm of ³²P-labelled IAP cDNA probes (rat NAIP, rat XIAP or human HIAP-2) added to the prehybridization buffer. The membranes
5 were then washed twice with SSC (300 mM NaCl, 30 mM sodium citrate) in 0.1% SDS for 20 min at room temperature and twice with SSC (30 mM NaCl, 3 mM sodium citrate) in 0.1% SDS for 20 min at 55°C and exposed to X-ray film at -80°C for visualization. Densitometric analysis of various IAPs and 28S rRNA band was performed with the Image Analysis Systems from Bio-Rad Laboratories. Data were normalized by the respective 28S
10 and expressed as a percentage of the control (defined as 100%).

Results

We observed the following.

1. Cisplatin induced a concentration-dependent increase in the incidence of apoptosis in cisplatin-sensitive (OV2008) but to a lesser extent in -resistant (C13) human ovarian
15 epithelial cells in vitro (Fig. 20). Similarly, Taxol also induced apoptosis in OV2008 cells, but to a lesser extent in the C13 cells (Fig. 21).
2. Basal XIAP and HIAP-2 protein contents were markedly higher in cisplatin-sensitive than -resistant cells. Taxol (0.04-1.0 µM) decreased XIAP and HIAP-2 protein levels in a concentration-dependent manner, the response being more pronounced in sensitive than
20 resistant cells (Fig. 22). A lower molecular weight (approx. 45 kDa) immunoreactive fragment of HIAP-2 was also evident in both the sensitive and resistant cells. The content of this fragment was increased in the C13 cells but decreased in OV2008 cells by Taxol (Fig. 22).
3. Whereas Taxol (0.2 µM) markedly suppressed HIAP-2 mRNA abundance in cisplatin-
25 sensitive cells (approx. 80%), it was ineffective in the resistant cells (Fig. 23).
4. TGFβ (20ng/ml) induced apoptosis in OV2008 but not in C13. Although its influence on XIAP protein content in cisplatin-resistant cells was only marginal, it markedly suppressed

- 36 -

the protein level of this IAP in the cisplatin-sensitive cells (Fig. 24A, 24B). TGF β (20 ng/ml) also decreased HIAP-2 mRNA in OV2008 but not C13 cells (Fig. 23).

Significant observations and possible applications.

Induction of apoptosis in human ovarian epithelial cancer cell by Taxol was
5 accompanied by suppressed IAP gene expression. Eventual loss of sensitivity of the cells to the chemotherapeutic agent may be associated with the decreased ability of the cell to express IAP genes. In drug-resistant cells, the decreased HIAP-2 protein content (in the face of an absence of noticeable change in HIAP-2 mRNA abundance) in the presence of Taxol was accompanied an increase in the intensity of a 45 kDa immunoreactive HIAP-2 protein band.
10 These observations lead us to believe that the 45 kDa protein is a proteolytic product of HIAP-2 and plays a role in the development of drug resistance. In addition, the sensitivity of the IAP family in these ovarian cancer cells to Taxol suggest possible novel sites for gene targeting in the development of new chemotherapeutic agents for the treatment of human ovarian epithelial cell cancer.

15 EXAMPLE 4: Accumulation of a 26 kDa Cleavage Protein in Astrocytoma Cells

Identification of a 26 kDa Cleavage Protein

A total protein extract was prepared from Jurkat and astrocytoma cells by sonicating them (X3 for 15 seconds at 4°C) in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM PMSF, 1 μ g/ml aprotinin, and 5 mM benzamidinc. Following sonication, the samples were
20 centrifuged (14,000 RPM in a micro centrifuge) for five minutes. 20 μ g of protein was loaded per well on a 10% SDS-polyacrylamide gel, electrophoresed, and electroblotted by standard methods to PVDF membranes. Western blot analysis, performed as described previously, revealed that the astrocytoma cell line (CCF-STTG1) abundantly expressed an anti-xiap reactive band of approximately 26 kDa, despite the lack of an apoptotic trigger
25 event (Fig. 12). In fact, this cell line has been previously characterized as being particularly resistant to standard apoptotic triggers.

A 26 kDa XIAP-reactive band was also observed under the following experimental conditions. Jurkat cells (a transformed human T cell line) were induced to undergo apoptosis by exposure to an anti-Fas antibody (1 μ g/ml). Identical cultures of Jurkat cells were

- 37 -

exposed either to: (1) anti-Fas antibody and cycloheximide (20 µg/ml), (2) tumor necrosis factor alpha (TNF-α, at 1,000 U/ml), or (3) TNF-α and cycloheximide (20 µg/ml). All cells were harvested 6 hours after treatment began. In addition, as a negative control, anti-Fas antibody was added to an extract after the cells were harvested. The cells were harvested in
5 SDS sample buffer, electrophoresed on a 12.5% SDS polyacrylamide gel, and electroblotted onto PVDF membranes using standard methods. The membranes were immunostained with a rabbit polyclonal anti-XIAP antibody at 1:1000 for 1 hour at room temperature. Following four 15 minute washes, a goat anti-rabbit antibody conjugated to horse-radish peroxidase was applied at room temperature for 1 hour. Unbound secondary antibody was washed away, and
10 chemiluminescent detection of XIAP protein was performed. The Western blot revealed the presence of the full-length, 55 kDa XIAP protein, both in untreated and treated cells. In addition, a novel, approximately 26 kDa XIAP-reactive band was also observed in apoptotic cell extracts, but not in the control, untreated cell extracts (Fig. 13).

Cleavage of XIAP occurs in a variety of cell types, including other cancer cell lines
15 such as HeLa. The expression of the 26 kDa XIAP cleavage product was demonstrated in HeLa cells as follows. HeLa cells were treated with either: (1) cyclohexamide (20 µg/ml), (2) anti-Fas antibody (1 µg/ml), (3) anti-Fas antibody (1 µg/ml) and cyclohexamide (20 µg/ml), (4) TNFα (1,000 U/ml), or (5) TNFα (1,000 U/ml) and cyclohexamide (20 µg/ml). All cells were harvested 18 hours after treatment began. As above, anti-Fas antibody was
20 added to an extract after the cells were harvested. HeLa cells were harvested, and the Western blot was probed under the same conditions as used to visualize XIAP-reactive bands from Jurkat cell samples. A 26 kDa XIAP band was again seen in the apoptotic cell preparations (Fig. 14). Furthermore, the degree of XIAP cleavage correlated positively with cellular exposure to apoptotic triggers. Treatment of HeLa cells with cycloheximide or
25 TNFα alone caused only minor apoptosis, and little cleavage product was observed. If the cells were treated with the anti-Fas antibody, a greater amount of cleavage product was apparent. These data indicate that XIAP is cleaved in more than one cell type and in response to more than one type of apoptotic trigger.

Time Course of Expression

- 38 -

The time course over which the 26 kDa cleavage product accumulates was examined by treating HeLa and Jurkat cells with anti-Fas antibody (1 µg/ml) and harvesting them either immediately, or 1, 2, 3, 5, 10, or 22 hours after treatment. Protein extracts were prepared and Western blot analysis was performed as described above. Both types of cells accumulated increasing quantities of the 26 kDa cleavage product over the time course examined (Figs. 15A and 15B).

Subcellular Localization of the 26 kDa XIAP Cleavage Product

In order to determine the subcellular location of the 26 kDa cleavage product, Jurkat cells were induced to undergo apoptosis by exposure to anti-Fas antibody (1 µg/ml) and were then harvested either immediately, 3 hours, or 7 hours later. Total protein extracts were prepared, as described above, from cells harvested at each time point. In order to prepare nuclear and cytoplasmic cell extracts, apoptotic Jurkat cells were washed with isotonic Tris buffered saline (pH 7.0) and lysed by freezing and thawing five times in cell extraction buffer (50 mM PIPES, 50 mM KCl, 5 mM EGTA, 2 mM MgCl₂, 1 mM DTT, and 20 µM cytochalasin B). Nuclei were pelleted by centrifugation and resuspended in isotonic Tris (pH 7.0) and frozen at -80°C. The cytoplasmic fraction of the extract was processed further by centrifugation at 60,000 RPM in a TA 100.3 rotor for 30 minutes. Supernatants were removed and frozen at -80°C. Samples of both nuclear and cytoplasmic fractions were loaded on a 12.5% SDS-polyacrylamide gel, and electroblotted onto PVDF membranes. Western blot analysis was then performed using either an anti-CPP32 antibody (Transduction Laboratories Lexington, KY; Fig. 16A) or the rabbit anti-XIAP antibody described above (Fig. 16B).

The anti-CPP32 antibody, which recognizes the CPP32 protease (also known as YAMA or Apopain) partitioned almost exclusively in the cytoplasmic fraction. The 55 kDa XIAP protein localized exclusively in the cytoplasm of apoptotic cells, in agreement with the studies presented above, where XIAP protein in normal, healthy COS cells was seen to localize, by immunofluorescence microscopy, to the cytoplasm. In contrast, the 26 kDa cleavage product localized exclusively to the nuclear fraction of apoptotic Jurkat cells. Taken together, these observations suggest that the anti-apoptotic component of XIAP could be the 26 kDa cleavage product, which exerts its influence within the nucleus.

- 39 -

In vitro Cleavage of XIAP protein and Characterization of the Cleavage Product

For this series of experiments, XIAP protein was labeled with ^{35}S using the plasmid pcDNA3-6myc-XIAP, T7 RNA polymerase, and a coupled transcription/translation kit (Promega, Madison, WI) according to the manufacturer's instructions. Radioactively labeled XIAP protein was separated from unincorporated methionine by column chromatography using Sephadex G-50TM. In addition, extracts of apoptotic Jurkat cells were prepared following treatment with anti-Fas antibody (1 $\mu\text{g}/\text{ml}$) for three hours. To prepare the extracts, the cells were lysed in Triton X-100 buffer (1% Triton X-100, 25 mM Tris HCl) on ice for two hours and then microcentrifuged for 5 minutes. The soluble extract was retained (and was labeled TX100). Cells were lysed in cell extraction buffer with freeze/thawing. The soluble cytoplasmic fraction was set aside (and labeled CEB). Nuclear pellets from the preparation of the CEB cytoplasmic fraction were solubilized with Triton X-100 buffer, microcentrifuged, and the soluble fractions, which contains primarily nuclear DNA, was retained (and labeled CEB-TX100). Soluble cell extract was prepared by lysing cells with NP-40 buffer, followed by microcentrifugation for 5 minutes (and was labeled NP-40). *In vitro* cleavage was performed by incubating 16 μl of each extract (CEB, TX-100, CEB-TX100, and NP-40) with 4 μl of *in vitro* translated XIAP protein at 37°C for 7 hours. Negative controls, containing only TX100 buffer or CEB buffer were also included. The proteins were separated on a 10% SDS-polyacrylamide gel, which was dried and exposed to X-ray film overnight.

In vitro cleavage of XIAP was apparent in the CEB extract. The observed molecular weight of the cleavage product was approximately 36 kDa (Fig. 17). The 10 kDa shift in the size of the cleavage product indicates that the observed product is derived from the amino-terminus of the recombinant protein, which contains six copies of the myc epitope (10 kDa). It thus appears that the cleavage product possesses at least two of the BIR domains, and that it is localized to the nucleus.

EXAMPLE 5: CHARACTERIZATION OF IAP ACTIVITY AND INTRACELLULAR LOCALIZATION STUDIES

The ability of IAPs to modulate apoptosis can be defined *in vitro* systems in which alterations of apoptosis can be detected. Mammalian expression constructs carrying IAP

- 40 -

cDNAs, which are either full-length truncated, or antisense constructs can be introduced into cell lines such as CHO, NIH 3T3, HL60, Rat-1, or Jurkat cells. In addition, SF21 insect cells may be used, in which case the IAP gene is preferentially expressed using an insect heat shock promoter. Following transfection, apoptosis can be induced by standard methods, which include serum withdrawal, or application of staurosporine, menadione (which induces apoptosis via free radical formation), or anti-Fas antibodies. As a control, cells are cultured under the same conditions as those induced to undergo apoptosis, but either not transfected, or transfected with a vector that lacks an IAP insert. The ability of each IAP related construct to inhibit or enhance apoptosis upon expression can be quantified by calculating the survival index of the cells, *i.e.*, the ratio of surviving transfected cells to surviving control cells. These experiments can confirm the presence of apoptosis inhibiting activity and, as discussed below, can also be used to determine the functional region(s) of an IAP which may be employed to achieve enhancement of apoptosis. These assays may also be performed in combination with the application of additional compounds in order to identify compounds that enhance apoptosis via IAP expression.

EXAMPLES 6: CELL SURVIVAL FOLLOWING TRANSFECTION WITH IAP CONSTRUCTS AND INDUCTION OF APOPTOSIS

Specific examples of the results obtained by performing various apoptosis suppression assays are shown in Figs. 10A to 10D. For example, CHO cell survival following transfection with one of six constructs and subsequent serum withdrawal is shown in Fig. 10A. The cells were transfected using Lipofectace™ with 2 µg of one of the following recombinant plasmids: pCDNA36myc-xiap (XIAP), pCDNA3-6myc-hiap-1 (HIAP-1), pCDNA3-6myc-hiap-2 (HIAP-2), pCDNA3-bcl-2 (BCL-2), pCDNA3-HA-smn (SMN), and pCDNA3-6myc (6-myc). Oligonucleotide primers were synthesized to allow PCR amplification and cloning of the XIAP, HIAP-1, and HIAP-2 ORFs in pCDNA3 (Invitrogen). Each construct was modified to incorporate a synthetic myc tag encoding six repeats of the peptide sequence MEQKLISEEDL (SEQ ID NO: 17), thus allowing detection of myc-IAP fusion proteins via monoclonal anti-myc antiserum (Egan *et al.*, Nature 363: 45-51, 1993). Triplicate samples of cell lines in 24-well dishes were washed 5 times with serum free media and maintained in serum free conditions during the course of the experiment.

- 41 -

Cells that excluded trypan blue, and that were therefore viable, were counted with a hemocytometer immediately, 24 hours, 48 hours, and 72 hours, after serum withdrawal. Survival was calculated as a percentage of the initial number of viable cells. In this experiment and those presented in Figs. 10B and 10D, the percentage of viable cells shown
5 represents the average of three separate experiments performed in triplicate, +/- average deviation.

The survival of CHO cells following transfection (with each one of the six constructs described above) and exposure to menadione is shown in Fig. 10B. The cells were plated in 24-well dishes, allowed to grow overnight, and then exposed to 20 μ M menadione for 1.5
10 hours (Sigma Chemical Co., St. Louis, MO). Triplicate samples were harvested at the time of exposure to menadione and 24 hours afterward, and survival was assessed by trypan blue exclusion.

The survival of Rat-1 cells following transfection (with each one of the six constructs described above) and exposure to staurosporine is shown in Fig. 10C. Rat-1 cells were
15 transfected and then selected in medium containing 800 μ g/ml G418 for two weeks. The cell line was assessed for resistance to staurosporine-induced apoptosis (1 μ M) for 5 hours. Viable cells were counted 24 hours after exposure to staurosporine by trypan blue exclusion. The percentage of viable cells shown represents the average of two experiments, +/- average deviation.

20 The Rat-1 cell line was also used to test the resistance of these cells to menadione (Fig. 10D) following transfection with each of the six constructs described above. The cells were exposed to 10 μ M menadione for 1.5 hours, and the NUMBER of viable cells was counted 18 hours later.

25 EXAMPLE 7: COMPARISON OF CELL SURVIVAL FOLLOWING TRANSFECTION WITH FULL-LENGTH VS. PARTIAL IAP CONSTRUCTS

In order to investigate the mechanism whereby human IAPs, including XIAP, HIAP-1, and HIAP-2, afford protection against cell death, expression vectors were constructed that contained either: (1) full-length IAP cDNA (as described above), (2) a portion of an IAP gene that encodes the BIR domains, but not the RZF, or (3) a portion of an IAP gene that
30 encodes the RZF, but not the BIR domains. Human and murine XIAP cDNAs were tested by

- 42 -

transient or stable expression in HcLa, Jurkat, and CHO cell lines. Following transfection, apoptosis was induced by serum withdrawal, application of menadione, or application of an anti-Fas antibody. Cell death was then assessed, as described above, by trypan blue exclusion. As a control for transfection efficiency, the cells were co-transfected with a β -gal expression construct. Typically, approximately 20% of the cells were successfully transfected.

When CHO cells were transiently transfected, constructs containing full-length human or mouse xiap cDNAs conferred modest but definite protection against cell death. In contrast, the survival of CHO cells transfected with constructs encoding only the BIR domains (*i.e.*, lacking the RZF domain) was markedly enhanced 72 hours after serum deprivation. Furthermore, a large percentage of cells expressing the BIR domains were still viable after 96 hours, at which time no viable cells remained in the control, *i.e.* non-transfected, cell cultures, and less than 5% of the cells transfected with the vector only, *i.e.*, lacking a cDNA insert, remained viable. Deletion of any of the BIR domains results in the complete loss of apoptotic suppression, which is reflected by a decrease in the percentage of surviving CHO cells to control levels within 72 hours of serum withdrawal.

Stable pools of transfected CHO cells, which were maintained for several months under G418 selection, were induced to undergo apoptosis by exposure to 10 μ M menadione for 2 hours. Among the CHO cells tested were those that were stably transfected with: (1) full-length murine XIAP cDNA (MIAP), (2) full-length XIAP cDNA (XIAP), (3) full-length BCL-2 cDNA (BCL-2), (4) cDNA encoding the three BIR domains (but not the RZF) of murine XIAP (BIR), and (5) cDNA encoding the RZF (but not BIR domains) of M-XIAP (RZF). Cells that were non-transfected (CHO) or transfected with the vector only (pcDNA3), served as controls for this experiment. Following exposure to 10 μ M menadione, the transfected cells were washed with phosphate buffered saline (PBS) and cultured for an additional 24 hours in menadione-free medium. Cell death was assessed, as described above, by trypan blue exclusion. Less than 10% of the non-transfected or vector-only transfected cells remained viable at the end of the 24 hour survival period. Cells expressing the RZF did not fare significantly better. However, expression of full-length murine XIAP, human XIAP, or BCL-2, and expression of the BIR domains, enhanced cell survival. When the concentration of menadione was increased from 10 μ M to 20 μ M (with all other conditions

- 43 -

of the experiment being the same as when 10 μ M menadione was applied), the percentage of viable CHO cells that expressed the BIR domain cDNA construct was higher than the percentage of viable cells that expressed either full-length murine XIAP or BCL-2.

EXAMPLE 8: ANALYSIS OF THE SUBCELLULAR LOCATION OF EXPRESSED RZF AND BIR DOMAINS

The assays of cell death described above indicate that the RZF acts as a negative regulator of the anti-apoptotic function of IAPs. One way in which the RZF, and possibly other IAP domains, may exert their regulatory influence is by altering the expression of genes, whose products function in the apoptotic pathway.

10 In order to determine whether the subcellular locations of expressed RZF and BIR domains are consistent with roles as nuclear regulatory factors, COS cells were transiently transfected with the following four constructs, and the expressed polypeptide was localized by immunofluorescent microscopy: (1) pcDNA3-6myc-XIAP, which encodes all 497 amino acids of SEQ ID NO: 4, (2) pcDNA3-6myc-m-XIAP, which encodes all 496 amino acids of
15 mouse XIAP (SEQ ID NO: 10), (3) pcDNA3-6myc-mxiap-BIR, which encodes amino acids 1 to 341 of m-XIAP, and (4) pcDNA3-6myc-mxiap-RZF, which encodes amino acids 342-496 of murine XIAP. The cells were grown on multi-well tissue culture slides for 12 hours, and then fixed and permeabilized with methanol. The constructs used (here and in the cell death assays) were tagged with a human Myc epitope tag at the N-terminus. Therefore, a
20 monoclonal anti-Myc antibody and a secondary goat anti-mouse antibody, which was conjugated to FITC, could be used to localize the expressed products in transiently transfected COS cells. Full-length XIAP and MIAP were located in the cytoplasm, with accentuated expression in the peri-nuclear zone. The same pattern of localization was observed when the cells expressed a construct encoding the RZF domain (but not the BIR
25 domains). However, cells expressing the BIR domains (without the RZF) exhibited, primarily, nuclear staining. The protein expressed by the BIR domain construct appeared to be in various stages of transfer to the nucleus.

These observations are consistent with the fact that, as described below, XIAP is cleaved within T cells that are treated with anti-Fas antibodies (which are potent inducers of

apoptosis), and its N-terminal domain is translocated to the nucleus. As noted in Example 2, HIAP-2 appears to undergo a similar cleavage event.

EXAMPLE 9: TESTING OF ANTISENSE OLIGONUCLEOTIDES:

1. *Complete panel of adenovirus constructs.* The panel may consist of approximately four
5 types of recombinant virus. A) Sense orientation viruses for each of the IAP or NAIP open
reading frames: XIAP, HIAP-1, HIAP-2, and NAIP. These viruses are designed to
massively overexpress the recombinant protein in infected cells. B) Antisense orientation
viruses in which the viral promoter drives the synthesis of an mRNA of opposite polarity to
the IAP mRNA, thereby shutting off host cell synthesis of the targeted protein coding region.
10 XIAP, HIAP-1, HIAP-2, and NAIP "antisense" constructs required. C) Sub-domain
expression viruses. These constructs express only a partial IAP protein in infected cells. Our
results indicate that deletion of the zinc finger of XIAP renders the protein more potent in
protecting cell against apoptotic triggers. This data also indicates that expression of the zinc
finger alone will indicate apoptosis by functioning as a dominant-negative repressor of XIAP
15 function. XIAP-ΔZF and XIAP-ΔBIR viruses required. D) Control viruses. Functional
analysis of the IAPs requires suitable positive and negative controls for comparison. BCL-2
sense, BCL-2 antisense, p53 sense, and Lac Z (negative control) viruses may be utilized.
2. *Confirmation of recombinant adenovirus function.* Verification of the sense adenovirus
function involves infection of tissue culture cells and determination of protein expression
20 levels. We have performed western blot analysis of several of the recombinant adenoviruses,
including NAIP, XIAP and XIAP-ΔRZF. The remaining viruses may be readily
assessed for protein expression using the polyclonal IAP antibodies. Functional analysis of
the antisense viruses may be done at the RNA level using either northern blots of total RNA
harvested from infected tissue culture cells or ribonuclease protection assays. Western blot
25 analysis of infected cells will be used to determine whether the expressed antisense RNA
interferes with IAP expression in the host cell.
3. *Documentation that IAP overexpression results in increased drug resistance.* We have
optimized cell death assays to allow high through-put of samples with minimal sample

- 45 -

variation. Testing of the sense IAP adenoviruses for their ability to alter drug sensitivity of breast and pancreatic adenocarcinoma cell lines may be accomplished as follows. Cancer cell lines are infected with the recombinant viruses, cultured for 5 days, then subdivided into 24 well plates. Triplicate cell receive increasing concentrations of the anti-cancer drug under investigation. Samples are harvested at 24, 48, and 72 hours post exposure, and assayed for the number of viable cells in the well. The dose response curve is then compared to uninfected and control virus (both positive and negative) infected cells. One may document a dramatic increase in the relative resistance of the cancer cell lines when infected with the sense viruses, confirming our hypothesis that overexpression of the IAP proteins contributes to the anti-apoptotic phenotype of cancer cells. Initial experiments utilize the chemotherapeutic drugs doxorubicin and adriamycin.

4. *Documentation that antisense IAP overexpression results in increased drug sensitivity.*

Having confirmed that IAP overexpression renders cancer cell more resistant to chemotherapeutic drugs, one may examine whether the antisense adenoviruses render the same cells more sensitive. The effectiveness of antisense IAP viruses relative to antisense BCL-2 virus will also be assessed as a crucial milestone.

5. *Identification of antisense oligonucleotides.* Concomitant to the adenovirus work, we have designed a series of antisense oligonucleotides to various regions of each of the IAPs. A generally accepted model of how antisense oligonucleotides function proposes that the formation of RNA/DNA duplexes in the nucleus activates cellular RnaseH enzymes which then enzymatically degrade the mRNA component of the hybrid. Virtually any region of the mRNA can be targeted, and therefore choosing an appropriate sequence to target is somewhat empirical. Many factors, including secondary structure of the target mRNA and the binding affinity of the targeted sequence determine whether a particular oligonucleotide will be effective, necessitating several oligonucleotides for each IAP. Five oligonucleotides have been made for each IAP mRNA based on the available computer algorithms for predicting binding affinities and mRNA secondary structures. These and other oligonucleotides may be tested for their ability to target their respective mRNAs for degradation using northern blot analysis.

- 46 -

6. *Optimization of oligonucleotides.* A secondary round of oligonucleotides may be made when effective target regions have been identified. These oligonucleotides target sequences in the immediate vicinity of the most active antisense oligonucleotides identified using methods such as those provided above. A second round of testing by northern blot analysis
5 may be required.

7. *Testing antisense oligonucleotides in vitro.* Following successful identification and optimization of targeting oligonucleotides, one may test these in the tissue culture model system using the optimal cell lines such as those described in the cancer survey described herein. Experimental procedures may parallel those used in the recombinant antisense
10 adenovirus work. Negative control oligonucleotides with miss-match sequences are used to establish base line or non-specific effects. Assisted transfection of the oligonucleotides using cationic lipid carriers may be compared to unassisted transfection. Confirmation of the effectiveness of specific antisense oligonucleotides prompts synthesis of oligonucleotides with modified phosphodiester linkages, such as phosphorothioate or methylimino substituted
15 oligonucleotides. These may also be tested *in vitro*.

8. *Animal modeling of antisense oligonucleotide therapies.*
Animal modeling of the effectiveness of the antisense IAP approach is described here. Cell lines are routinely assessed for their tumorigenic potential in "nude" mice, a hairless strain of mouse that is immunocompromised (lacks a functional thymus), and thus extremely
20 susceptible to developing tumors. In the nude mouse assay, cancer cells are grown in tissue culture and then injected under the skin at multiple sites. The frequency with which these cells give rise to palpable tumors within a defined period of time provides an index of the tumorigenic potential of the cell line in the absence of interference by a functional immune system. Preliminary assessment of an antisense IAP therapeutic involves injection of cancer
25 cells infected with the recombinant adenoviruses (sense, antisense, and control viruses) under the skin, and the tumorigenic index compared to that of untreated cells. One may also use this model to assess the effectiveness of systemic administration of antisense oligonucleotides in increasing the efficacy of anti-cancer drugs in the nude mouse model. Phosphorothioate or methylimino substituted oligonucleotides will be assessed at this stage.

This type of antisense oligonucleotide has demonstrated enhanced cell permeability and slower clearance rates from the body in experimental animal models.

EXAMPLE 10: ADDITIONAL APOPTOSIS ASSAYS

Specific examples of apoptosis assays are also provided in the following references.

- 5 Assays for apoptosis in lymphocytes are disclosed by: Li *et al.*, Science 268: 429-431, 1995; Gibellini *et al.*, Br. J. Haematol. 89: 24-33, 1995; Martin *et al.*, J. Immunol. 152: 330-342, 1994; Terai *et al.*, J. Clin. Invest. 87: 1710-1715, 1991; Dhein *et al.*, Nature 373: 438-441, 1995; Katsikis *et al.*, J. Exp. Med. 1815: 2029-2036, 1995; Westendorp *et al.*, Nature 375: 497-500, 1995; DeRossi *et al.*, Virology 198: 234-244, 1994.
- 10 Assays for apoptosis in fibroblasts are disclosed by: Vossheck *et al.*, Int. J. Cancer 61: 92-97, 1995; Goruppi *et al.*, Oncogene 9: 1537-1544, 1994; Fernandez *et al.*, Oncogene 9: 2009-2017, 1994; Harrington *et al.*, EMBO J., 13: 3286-3295, 1994; Itoh *et al.*, J. Biol. Chem. 268: 10932-10937, 1993.
- Assays for apoptosis in neuronal cells are disclosed by: Melino *et al.*, Mol. Cell. Biol. 14: 6584-6596, 1994; Rosenbaum *et al.*, Ann. Neurol. 36: 864-870, 1994; Sato *et al.*, J. Neurobiol. 25: 1227-1234, 1994; Ferrari *et al.*, J. Neurosci. 1516: 2857-2866, 1995; Talley *et al.*, Mol. Cell. Biol. 15: 2359-2366, 1995; Walkinshaw *et al.*, J. Clin. Invest. 95: 2458-2464, 1995.
- 15 Assays for apoptosis in insect cells are disclosed by: Clem *et al.*, Science 254: 1388-1390, 1991; Crook *et al.*, J. Virol. 67: 2168-2174, 1993; Rabizadeh *et al.*, J. Neurochem. 61: 2318-2321, 1993; Birnbaum *et al.*, J. Virol. 68: 2521-2528, 1994; Clem *et al.*, Mol. Cell. Biol. 14: 5212-5222, 1994.

EXAMPLE 11: CONSTRUCTION OF A TRANSGENIC ANIMAL

- Characterization of IAP and NAIP genes provided information that necessary for
- 25 generation IAP and NAIP transgenic animal models to be developed by homologous recombination (for knockouts) or transfection (for expression of IAP or NAIP fragments, antisense nucleic acids, or increased expression of wild-type or mutant IAPs or NAIP). Such a model may be a mammalian animal, *e.g.*, a mouse, and is useful for the identification of

cancer therapeutics alone or in combination with cancer inducing cells or agents, or when such mice are crossed with mice genetically predisposed to cancers.

The preferred transgenic animal overexpression in IAP or NAIP and has a predisposition to cancer. This mouse is particularly useful for the screening of potential
5 cancer therapeutics.

EXAMPLE 12: IAP OR NAIP PROTEIN EXPRESSION

IAP and NAIP genes and fragments thereof (*i.e.*, RZF fragments) may be expressed in both prokaryotic and eukaryotic cell types. If an IAP or NAIP fragment enhances apoptosis, it may be desirable to express that protein under control of an inducible promoter.

10 In general, IAPs and NAIP, and fragments thereof, may be produced by transforming a suitable host cell with all or part of the IAP-encoding or NAIP-encoding cDNA fragment that has been placed into a suitable expression vector.

Those skilled in the art of molecular biology will understand that a wide variety of expression systems may be used to produce the recombinant protein. The precise host cell
15 used is not critical to the invention, although cancer cells are preferable. The IAP protein may be produced in a prokaryotic host (*e.g.*, *E. coli*) or in a eukaryotic host (*e.g.*, *S. cerevisiae*, insect cells such as Sf21 cells, or mammalian cells such as COS-1, NIH 3T3, or HeLa cells, or other highly proliferative cell types). These cells are publically available, for example, from the American Type Culture Collection, Rockville, MD; see also Ausubel *et al.*, *supra*). The method of transduction and the choice of expression vehicle will depend on
20 the host system selected. Transformation and transfection methods are described, *e.g.*, in Ausubel *et al.* (*supra*), and expression vehicles may be chosen from those provided, *e.g.*, in Cloning Vectors: A Laboratory Manual (P.H. Pouwels *et al.*, 1985, Supp. 1987).

Polypeptides of the invention, particularly short IAP fragments, can also be produced
25 by chemical synthesis (*e.g.*, by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL). These general techniques of polypeptide expression and purification can also be used to produce and isolate useful IAP fragments or analogs, as described herein.

EXAMPLE 13: ANTI-IAP AND ANTI-NAIP ANTIBODIES

In order to generate IAP-specific and NAIP-specific antibodies, an IAP or NAIP coding sequence (e.g., amino acids 180-276) can be expressed as a C-terminal fusion with glutathione S-transferase (GST; Smith *et al.*, Gene 67: 31-40, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione, and cleaved with
5 thrombin (at the engineered cleavage site), and purified to the degree required to successfully immunize rabbits. Primary immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and immunoprecipitation analyses using the thrombin-cleaved IAP fragment of the GST-IAP and GST-NAIP fusion proteins. Immune
10 sera are affinity purified using CNBr-Sepharose-coupled IAP protein. Antiserum specificity is determined using a panel of unrelated GST proteins (including GSTp53, Rb, HPV-16 E6, and E6-AP) and GST-trypsin (which was generated by PCR using known sequences).

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique hydrophilic regions of IAP or NAIP may be generated and coupled to
15 keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity is tested by ELISA and Western blotting using peptide conjugates, and by Western blotting and immunoprecipitation using IAP or NAIP expressed as a GST fusion protein.

20 Alternatively, monoclonal antibodies may be prepared using the IAP or NAIP proteins described above and standard hybridoma technology (see, e.g., Kohler *et al.*, Nature 256: 495, 1975; Kohler *et al.*, Eur. J. Immunol. 6: 511, 1976; Kohler *et al.*, Eur. J. Immunol. 6:292, 1976; Hammerling *et al.*, In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, New York, NY, 1981; Ausubel *et al.*, *supra*). Once produced, monoclonal antibodies are
25 also tested for specific IAP or NAIP recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel *et al.*, *supra*).

Antibodies that specifically recognize IAPs or NAIP or fragments thereof, such as those described herein containing one or more BIR domains (but not a ring zinc finger domain), or that contain a ring zinc finger domain (but not a BIR domain) are considered
30 useful in the invention. They may, for example, be used in an immunoassay to monitor IAP or NAIP expression levels or to determine the subcellular location of an IAP or NAIP (or

- 50 -

fragment thereof) produced by a mammal. Antibodies that inhibit the 26 kDa IAP cleavage product described herein (which contains at least one BIR domain) may be especially useful in inducing apoptosis in cells undergoing undesirable proliferation.

Preferably, antibodies of the invention are produced using IAP or NAIP sequence that
5 does not reside within highly conserved regions, and that appears likely to be antigenic, as analyzed by criteria such as those provided by the Peptide structure program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (CABIOS 4: 181, 1988).
Specifically, these regions, which are found between BIR1 and BIR2 of all IAPs, are: from
10 amino acid 99 to amino acid 170 of HIAP-1, from amino acid 123 to amino acid 184 of HIAP-2, and from amino acid 116 to amino acid 133 of either XIAP or m-XIAP. These fragments can be generated by standard techniques, e.g., by the PCR, and cloned into the pGEX expression vector (Ausubel *et al.*, *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel *et al.* (*supra*). In
15 order to minimize the potential for obtaining antisera that is non-specific, or exhibits low-affinity binding to IAP, two or three fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in series, preferably including at least three booster injections.

20 **EXAMPLE 14: IDENTIFICATION OF MOLECULES THAT MODULATE THE
EXPRESSION OR BIOLOGICAL ACTIVITY OF AN IAP OR NAIP GENE**

IAP and NAIP cDNAs facilitate the identification of molecules that decrease IAP or NAIP expression or otherwise enhance apoptosis normally blocked by these polypeptides. Such compounds are highly useful as, for example, chemotherapeutic agents to destroy a cancer cell, or to reduce the growth of a cancer cell, where the cancer cell is one, as is
25 described herein, with an elevated level of an IAP or NAIP polypeptide.

In one approach, candidate molecules are added, in varying concentration, to the culture medium of cells expressing IAP or NAIP mRNA. IAP or NAIP expression is then measured, for example, by Northern blot analysis (Ausubel *et al.*, *supra*) using an IAP or NAIP cDNA, or cDNA fragment, as a hybridization probe. The level of IAP or NAIP
30 expression in the presence of the candidate molecule is compared to the level of IAP or NAIP

- 51 -

expression in the absence of the candidate molecule, all other factors (*e.g.*, cell type and culture conditions) being equal.

The effect of candidate molecules on IAP- or NAIP-mediated apoptosis may, instead, be measured at the level of protein or the level of polypeptide fragments of IAP or NAIP polypeptides using the general approach described above with standard polypeptide detection techniques, such as Western blotting or immunoprecipitation with an IAP or NAIP-specific antibodies (for example, the antibodies described herein).

Compounds that modulate the level of a IAP or NAIP polypeptide may be purified, or substantially purified, or may be one component of a mixture of compounds such as an extract or supernatant obtained from cells (Ausubel *et al.*, *supra*). In an assay of a mixture of compounds, IAP or NAIP polypeptide expression is tested against progressively smaller subsets of the compound pool (*e.g.*, produced by standard purification techniques such as HPLC or FPLC) until a single compound or minimal number of effective compounds is demonstrated to modulate IAP or NAIP expression.

Compounds may also be screened for their ability to modulate the biological activity of an IAP or NAIP polypeptide by, for example, an ability to enhance IAP- or NAIP-mediated apoptosis. In this approach, the degree of apoptosis in the presence of a candidate compound is compared to the degree of apoptosis in its absence, under equivalent conditions. Again, the screen may begin with a pool of candidate compounds, from which one or more useful modulator compounds are isolated in a step-wise fashion. Apoptosis activity may be measured by any standard assay, for example, those described herein.

Another method for detecting compounds that modulate the expression or biological activity of an IAP or a NAIP polypeptide is to screen for compounds that interact physically with a given IAP polypeptide. These compounds may be detected by adapting two hybrid systems known in the art. These systems detect protein interactions using a transcriptional activation assay and are generally described by Gyuris *et al.* (Cell 75: 791-803, 1993) and Field *et al.* (Nature 340: 245-246, 1989), and are commercially available from Clontech (Palo Alto, CA). In addition, PCT Publication WO 95/28497 describes a two hybrid system in which proteins involved in apoptosis, by virtue of their interaction with BCL-2, are detected. A similar method may be used to identify proteins and other compounds that interact with IAP or NAIP polypeptides.

- 52 -

Compounds or molecules that function as modulators of IAP-mediated cell death may include peptide and non-peptide molecules such as those present in cell extracts, mammalian serum, or growth medium in which mammalian cells have been cultured. In addition, compounds previously known for their abilities to modulate apoptosis in cancer cells may be tested for an ability to modulate expression of an IAP molecule.

TABLE 2

OLIGONUCLEOTIDE PRIMERS FOR THE SPECIFIC
RT-PCR AMPLIFICATION OF IAP GENES

10	IAP Gene	Forward Primer (nucleotide position*)	Reverse Primer (nucleotide position*)	Size of Product (bp)
	h-XIAP	p2415 (876-896)	p2449 (1291-1311)	435
	m-XIAP	p2566 (458-478)	p2490 (994-1013)	555
	h-HIAP 1	p2465 (827-847)	p2464 (1008-1038)	211
	m-HIAP 1	p2687 (747-767)	p2684 (1177-1197)	450
15	HIAP2	p2595 (1562-1585)	p2578 (2339-2363)	801& 618@
	m-HIAP2	p2693 (1751-1772)	p2734 (2078-2100)	349

* Nucleotide position as determined from Figs. 1-4 for each IAP gene

&PCR product size of hiap2a

@ PCR product size of hiap2b

20 **EXAMPLE 15: ROLE OF IAPs IN HUMAN OVARIAN CANCER RESISTANCE TO CISPLATIN**

Ovarian epithelial cancer cell apoptosis has been demonstrated to be involved in cisplatin-induced cell death (Havrilesky *et al.*, Obstet. Gynecol. 85: 1007-1010, 1995; Anthoney *et al.*, Cancer Res. 56: 1374-1381, 1996). The action of cisplatin is thought to involve the formation of inter and intra-strand DNA crosslinks (Sherman *et al.*, Science 230: 412-417, 1985) although the events leading to cell death after cisplatin treatment is unclear. If IAPs are indeed key elements in the regulation of apoptosis in ovarian cancer cells, one

- 53 -

would expect that down-regulation of this anti-apoptotic protein would result in cell death. To test this, cisplatin-sensitive human ovarian surface epithelial cells (OV2008) were infected with either adenoviral XIAP antisense, adenoviral HIAP-2 antisense, or the empty vector with LacZ (as control) for up to 60 hours, at which time changes in cell morphology, apoptotic cell number, cell viability, and total cell number were determined. The full length sense and antisense constructs of XIAP and HIAP-2 were prepared as briefly described hereafter. To construct the adenoviruses, the open reading frame for XIAP and HIAP-2 were PCR amplified with primers corresponding to the amino and carboxy terminus. These PCR products were cloned in the pCR2.1 vector (InvitroGen, Carlsbad, CA), and sequenced. The ORFs were then excised with EcoRI digestion, blunt ended with Klenow fragment, and ligated into Swal digested pAdex1CAwt cosmid DNA. Packaging was performed with Promega (Madison, WI) cosmid packaging extracts and used to infect *E. coli*. Colonies were picked and screened for the presence of the insert in both the sense and antisense orientation relative to the chicken B-actin (CA) promoter. CsCl purified cosmid DNA was co-transfected with wild-type adenovirus DNA, which contains the terminal protein complexed to the ends of the DNA. Wild type adenovirus DNA was cut with NsiI such that only homologous recombinant with the cosmid DNA generated infectious adenovirus DNA. The final recombinant adenovirus contains a linear, double stranded genome of 44,820 bp plus the insert size (approximately 1,500 for XIAP, approximately 1,800 for HIAP-2).

Cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) ovarian epithelial cancer cells were infected with adenovirus [multiplicity of infection (MOI) = 5 (1X); MOI = 10 (2X)] containing antisense XIAP or HIAP-2 cDNA, or vector (control) for 60 hours. Cells were then trypsinized and total cell number was determined with haemocytometry while cell viability was determined by the trypan blue dye exclusion test. XIAP antisense infection of OV2008 cells significantly increased the percentage of dead cells compared to control (vector, $p < 0.001$), as determined by trypan blue exclusion tests (Fig. 25, top left panel). Although there appeared also to be a slight increase in percentage of dead cells with HIAP-2 antisense infection of OV2008 cells, it was not statistically significant (Fig. 25, top left panel; $p > 0.05$). Infection of the cisplatin-resistant variant of OV2008 cells (C13) with antisense of XIAP but not of HIAP-2 also significantly, though to a lesser extent, decreased cell viability (Fig. 25, top right panel). The cell death induced in both OV2008 and C13 by XIAP

antisense was also accompanied by decreases in total cell number, with the effect of the antisense infection being more pronounced in the cisplatin-sensitive cells (Fig. 25, bottom two panels).

- In addition, 60 hours of adenoviral XIAP antisense infection of OV2008 decreased XIAP protein content and induced extensive cell detachment, as is shown in Fig. 26A (black arrows in left "b" photograph). Nuclear fragmentation (Fig. 26B, white arrows in photographs "b" and "d") and increased the number of apoptotic cells as well as the abundance of apoptotic bodies (Fig 26B: photographs "b" and "d" compared to "a" and "c") is also induced in OV2008 cells following 60 hours of infection with adenovirus XIAP antisense. For nuclear staining, cells were fixed in 4% formalin (in PBS, room temp., 10 min.) and washed in PBS. The washed cells were then resuspended in Hoechst staining solution (0.1 μ g Hoechst 33248/ml PBS, 10 min.), washed again, and spotted onto slides for microscopy. Nuclear staining was observed and photographed using a Zeiss fluorescence microscope. Cells with typical apoptotic nuclear morphology were identified and counted, using randomly selected fields and numbered photographic slides to avoid bias during counting. Analysis of variance indicated that there was highly significant effects of the antisense on XIAP protein content ($p < 0.001$; Fig. 26D and 26E) and apoptosis ($p < 0.001$; Fig 26C). Indeed, infection of these cells with a higher titre of the adenoviral anti-sense (MOI=10 (2X)) further increased the number of cells undergoing apoptosis (Fig. 26C).
- To study whether IAP expression is the target for the chemotherapeutic action of cisplatin, OV2008 cells were cultured in the absence and presence of cisplatin (10-30 μ M) for 24 hours, apoptosis and XIAP and HIAP-2 expression were assessed morphologically and by Western analysis, respectively. Like adenoviral XIAP antisense infection, the presence of cisplatin induced morphologic feature of apoptosis in OV2008 cells, including decreased cell volume, chromatin condensation and nuclear fragmentation (Fig. 27A, left two photographs), and apoptotic low molecular weight DNA fragmentation (Fig. 27B), and was accompanied by decreased IAP expression (Figs. 28A and 28B). The increase of apoptotic cell number in response to cisplatin was also concentration-dependent and was significant (50% vs. 2%; $p < 0.05$) even at a concentration of 10 μ M cisplatin (Fig. 27C).
- As shown in Figs. 28A and 28B, although both XIAP and HIAP-2 are present in the cisplatin-sensitive human ovarian surface epithelial cancer cell line OV2008 (protein sizes

- 55 -

55kDa and 68 kDa, respectively), their expression were down-regulated by cisplatin in a concentration-dependent manner. XIAP appearing more responsive to the anti-cancer agent. While XIAP protein content was decreased by almost 80% ($p < 0.01$) in the presence 20 μ M cisplatin, the decrease of HIAP-2 protein content was not suppressed by cisplatin (Figs. 28A and 28B).

The expression of XIAP and HIAP-2 in C13, the cisplatin-resistant variant of OV2008, was not suppressed by cisplatin (Figs. 28A and 28B), and no morphologic and biochemical changes characteristic of apoptosis could be detected (Figs. 27A and 27B). Although XIAP and HIAP-2 contents in C13 appeared to be higher in the presence of the anti-cancer agent, the differences were statistically non-significant ($p > 0.05$). Time course experiments on IAP expression demonstrated that the suppression of XIAP and HIAP-2 protein levels in OV2008 by cisplatin was time-dependent; a significant decrease was observed between 12-24 hours of culture (Figs. 29A and 29B). Expression of XIAP and HIAP-2 in C13 cells was not influenced by cisplatin, irrespective of the duration of treatment.

To determine if the observed XIAP responses in OV2008 and C13 cells were specific to this pair of cell lines, the influence of cisplatin in vitro on XIAP and HIAP-2 protein content in another cisplatin-sensitive ovarian surface epithelial cancer cell line (A2780s) and its cisplatin-resistant variant (A2780cp) was studied (Figs. 30A and 30B). Interestingly, whereas HIAP-2 expression in both the sensitive and resistant cells was not significantly altered by the presence of the cisplatin (30 μ M; Fig. 30B), XIAP protein content was decreased in A2780s (as in OV2008 cells) and not significantly altered in A2780cp (as in C13 cells) in the presence of the chemotherapeutic agent. Taken together, these data suggest that the apoptotic responsiveness of ovarian cancer cells to cisplatin may be related to the ability of the chemotherapeutic agent to down-regulate XIAP expression and that HIAP-2 may play a minor or no role in cisplatin-induced apoptosis.

To determine if XIAP expression is indeed the an important determinant in chemoresistance in human ovarian surface epithelial cancer, the influence of cisplatin on XIAP protein content and apoptosis in OV2008 cells following adenoviral XIAP sense infection was investigated. While cisplatin reduced XIAP protein content in OV2008 cells infected with the empty vector (Figs. 31C and 31D, vector plus cisplatin), overexpression of the protein with adenoviral sense XIAP cDNA 48 hrs prior to treatment with the

- 56 -

chemotherapeutic agent *in vitro* attenuated the cisplatin effects not only on XIAP protein expression (Figs. 31C and 31D) but also apoptotic nuclear fragmentation (Fig. 31A, "d" compared to "c") and number of apoptotic cells (Fig. 31B), suggesting that XIAP may be an important element in human ovarian epithelial cancer chemoresistance.

5 The *in vitro* studies with ovarian epithelial cancer cell lines strongly suggest an important role of IAPs, particularly of XIAP, in the control of apoptosis and tumor progression in human ovarian cancer. To determine if indeed IAPs are expressed in ovarian carcinoma and thus of clinical relevance, XIAP and HIAP-2 were immunolocalized in human ovarian surface epithelial tumors obtained as pathological samples from patients during
10 surgical debulking, using polyclonal antibodies (rabbit polyclonal anti-XIAP and HIAP-2 antibodies were prepared by immunization with human XIAP and HIAP-2 GST fusion protein) against human XIAP and HIAP-2, respectively (Figs. 32C and 32D, respectively). In addition, *in situ* TUNEL (described in Gavrieli *et al.*, J. Cell. Biol. 119: 493-501, 1992) and immunohistochemistry for PCNA (proliferating cell nuclear antigen: an auxiliary protein
15 of DNA polymerase α highly expressed as the G1/S interphase) were performed to examine if and how the expression of these IAPs relates to epithelial cell apoptosis and /or proliferation. Ovarian epithelial tumors exhibited considerable cellular heterogeneity (Fig. 32A) and PCNA positive cells were evident throughout the nucleus in the tumor section (Fig. 32B). In general, most of the cells were TUNEL negative (Fig. 32A), and the expression of
20 XIAP and HIAP-2 was highly correlated to the proliferative state of the cells and inversely related to epithelial cell death. XIAP and HIAP-2 immunoreactivity (Figs. 32C and 32D, respectively) specifically localized in the cytoplasm or the perinuclear region was highest in proliferatively active cells (PCNA positive) and was low or absent in apoptotic cells (TUNEL positive) occasionally found in the tumor specimens.

25 EXAMPLE 16: ADDITIONAL CANCER THERAPIES

Given the increased proliferation rate of cancer cells, it is preferable in anti-cancer therapeutic regimens to initiate treatment with an anti-cancer agent that will successfully inhibit the growth of the particular cancer of interest. One method to detect such an agent is
30 to excise proliferative cells from the cancer of interest, and determine the level of expression

- 57 -

and/or level of biological activity of each individual IAP or NAIP polypeptide, and compare these levels to the levels of these polypeptide in a similar cell type from an unaffected individual. For example, if an human female individual has breast cancer (or a neoplasm suspected of being cancerous), cells from the cancer collected, for example, during a biopsy
5 of the cancer, can be isolated and, if necessary, propagated in culture. The cells can then be analyzed for level of expression and/or level of biological activity of all of the IAP and NAIP polypeptides in the cell. The expression levels and/or biological activity levels of these polypeptides from the proliferating cells can be compared to the levels of expression and/or biological activity of these polypeptides from normal, healthy cells from a human female
10 individual. Preferably, the comparison is made between on affected (*i.e.*, abnormally proliferating) and healthy cells of the same individual (*e.g.*, cells taken from healthy breast tissue from the individual being tested. The level of expression and/or biological activity of each polypeptide in the affected cells is compared to its counterpart in the healthy cells. Any increase in any (or all) of the IAP or NAIP polypeptides is detected. The cancer is then
15 treated with a compound that decreases expression level or biological activity level of each particular elevated IAP or NAIP polypeptide. Methods for identifying such compounds are described above (see, *e.g.*, Example 14).

It will be understood that the individual undergoing such analysis and treatment may have already received treatment with an anti-cancer therapeutic agent. It will also be
20 understood that, in addition to targeting the levels of expression and/or biological activities of IAP and NAIP polypeptides, the anti-cancer compounds may also target these levels for other apoptosis-inhibiting polypeptides, such as BCL-2. For example, an individual with breast cancer whose proliferating cells have an increased level of XIAP compared to the level of XIAP in healthy breast cells may be treated with a compound (*e.g.*, cisplatin) plus a
25 compound that targets another IAP polypeptide, or that targets an NAIP polypeptide or a non-related apoptosis-inhibiting polypeptide, such as BCL-2).

One rapid method to determine expression levels of IAP and NAIP polypeptides is an ELISA assay using antibodies that specifically binds each of these polypeptides. Other methods include quantitative PCR and the various apoptosis assays described herein.

- 58 -

**EXAMPLE 17: ASSIGNMENT OF XIAP, HIAP-1, AND HIAP-2 TO
CHROMOSOMES XQ25 AND 11Q22-23 BY FLUORESCENCE IN SITU
HYBRIDIZATION (FISH)**

Fluorescence *in situ* hybridization (FISH) was used to identify the chromosomal
5 location of XIAP, HIAP-1 and HIAP-2.

A total of 101 metaphase spreads were examined with the XIAP probe, as described
above. Symmetrical fluorescent signals on either one or both homologs of chromosome
Xq25 were observed in 74% of the cells analyzed. Following staining with HIAP-1 and
HIAP-2 probes, 56 cells were analyzed and doublet signals in the region 11q22-23 were
10 observed in 83% of cells examined. The XIAP gene was mapped to Xq25 while the HIAP-1
and HIAP-2 genes were mapped at the border of 11q22 and 11q23 bands.

These experiments confirmed the location of the XIAP gene on chromosome Xq25.
No highly consistent chromosomal abnormalities involving band Xq25 have been reported so
far in any malignancies. However, deletions within this region are associated with a number
15 of immune system defects including X-linked lymphoproliferative disease (Wu *et al.*,
Genomics 17:163-170, 1993).

Cytogenetic abnormalities of band 11q23 have been identified in more than 50% of
infant leukemias regardless of the phenotype (Martinez-Climet *et al.*, Leukaemia 9: 1299-
1304, 1995). Rearrangements of the MLL Gene (mixed lineage leukemia or myeloid
20 lymphoid leukemia; Ziemer-van der Poel *et al.*, Proc. Natl. Acad. Sci. USA 88: 10735-
10739, 1991) have been detected in 80% of cases with 11q23 translocation, however patients
whose rearrangements clearly involved regions other than the MLL gene were also reported
(Kobayashi *et al.*, Blood 82: 547-551, 1993). Thus, the IAP genes may follow the BCL-2
paradigm, and would therefore play an important role in cancer transformation.

25 **Incorporation by Reference**

The following documents and all the references referred to herein are incorporated by
reference: U.S.S.N. 08/511,485, filed August 4, 1995; U.S.S.N. 08/576,956, filed December
22, 1995; PCT/IB96/01022, filed August 5, 1996; U.S.S.N. 60/017,354, filed April 26, 1996;
U.S.S.N. 60/030,931, filed November 15, 1996; U.S.S.N. 60/030,590, filed November 14,
30 1996; U.S.P.N. 5,576,208, issued November 19, 1996; and PCT Application No.

- 59 -

1B97/00142, filed January 17, 1997 claiming priority from UK 9601108.5, filed January 19, 1996.

Other Embodiments

In other embodiments, the invention includes use of any protein which is substantially
5 identical to a mammalian IAP polypeptides (Figs. 1-6; SEQ ID Nos: 3-14); such homologs
include other substantially pure naturally-occurring mammalian IAP proteins as well as
allelic variants; natural mutants; induced mutants; DNA sequences which encode proteins
and also hybridize to the IAP DNA sequences of Figs. 1-6 (SEQ ID NOS: 3-14) under high
stringency conditions or, less preferably, under low stringency conditions (*e.g.*, washing at
10 2X SSC at 40°C with a probe length of at least 40 nucleotides); and proteins specifically
bound by antisera directed to a IAP polypeptide. The term also includes chimeric
polypeptides that include a IAP portion.

The invention further includes use of analogs of any naturally-occurring IAP
polypeptide. Analogs can differ from the naturally-occurring IAP protein by amino acid
15 sequence differences, by post-translational modifications, or by both. Analogs of the
invention will generally exhibit at least 85%, more preferably 90%, and most preferably
95% or even 99% identity with all or part of a naturally occurring IAP amino acid sequence.
The length of sequence comparison is at least 15 amino acid residues, preferably at least 25
amino acid residues, and more preferably more than 35 amino acid residues. Modifications
20 include *in vivo* and *in vitro* chemical derivatization of polypeptides, *e.g.*, acetylation,
carboxylation, phosphorylation, or glycosylation; such modifications may occur during
polypeptide synthesis or processing or following treatment with isolated modifying enzymes.
Analogues can also differ from the naturally-occurring IAP polypeptide by alterations in
primary sequence. These include genetic variants, both natural and induced (for example,
25 resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by
site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular
Cloning: A Laboratory Manual, 2nd ed., CSH Press, 1989, or Ausubel *et al.*, *supra*). Also
included are cyclized peptides, molecules, and analogs which contain residues other than L-
amino acids, *e.g.*, D-amino acids or nonnaturally occurring or synthetic amino acids, *e.g.*, B
30 or γ amino acids. In addition to full-length polypeptides, the invention also includes IAP

- 60 -

polypeptide fragments. As used herein, the term "fragment," means at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of IAP polypeptides can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Preferable fragments or analogs used according to the methods of the invention are those which facilitate specific detection of an IAP nucleic acid or amino acid sequence in a sample to be diagnosed. Particularly useful IAP fragments for this purpose include, without limitation, the amino acid fragments shown in Table 2.

The methods of the invention may use antibodies prepared by a variety of methods. For example, the IAP or NAIP polypeptide, or antigenic fragments thereof, can be administered to an animal in order to induce the production of polyclonal antibodies. Alternatively, antibodies used as described herein may be monoclonal antibodies, which are prepared using hybridoma technology (see, e.g., Kohler *et al.*, Nature 256: 495-497, 1975; Kohler *et al.*, Eur. J. Immunol. 6: 511-519, 1976; Kohler *et al.*, Eur. J. Immunol. 6: 292-295, 1976; Hammerling *et al.*, In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, 1981; Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988). The invention features use of antibodies that specifically bind human or murine IAP or NAIP polypeptides, or fragments thereof. In particular the invention features "neutralizing" antibodies. By "neutralizing" antibodies is meant antibodies that interfere with any of the biological activities of IAP or NAIP polypeptides, particularly the ability of IAPs to inhibit apoptosis. The neutralizing antibody may reduce the ability of IAP polypeptides to inhibit polypeptides by, preferably 50%, more preferably by 70%, and most preferably by 90% or more. Any standard assay of apoptosis, including those described herein, by those incorporated by reference and those in the art, may be used to assess neutralizing antibodies.

In addition to intact monoclonal and polyclonal anti-IAP antibodies, the invention features use of various genetically engineered antibodies, humanized antibodies, and antibody fragments, including F(ab')₂, Fab', Fab, Fv and sFv fragments. Antibodies can be

- 61 -

humanized by methods known in the art, *e.g.*, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Fully human antibodies, such as those expressed in transgenic animals, are also features of the invention (Green *et al.*, Nature Genetics 7:13-21, 1994).

- 5 Ladner (U.S. Patent Nos. 4,946,778 and 4,704,692) describes methods for preparing single polypeptide chain antibodies. Ward *et al.* (Nature 341: 544-546, 1989) describe the preparation of heavy chain variable domains, which they term "single domain antibodies," which have high antigen-binding affinities. McCafferty *et al.* (Nature 348: 552-554, 1990) show that complete antibody V domains can be displayed on the surface of fd bacteriophage,
- 10 that the phage bind specifically to antigen, and that rare phage (one in a million) can be isolated after affinity chromatography. Boss *et al.* (U.S. Patent No. 4,816,397) describe various methods for producing immunoglobulins, and immunologically functional fragments thereof, which include at least the variable domains of the heavy and light chain in a single host cell. Cabilly *et al.* (U.S. Patent No. 4,816,567) describe methods for preparing chimeric
- 15 antibodies.

- 62 -

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: University of Ottawa

5 (ii) TITLE OF THE INVENTION: DETECTION AND MODULATION OF
IAPS AND NAIP FOR THE DIAGNOSIS
AND TREATMENT OF PROLIFERATIVE
DISEASE

(iii) NUMBER OF SEQUENCES: 17

10 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Clark & Elbing LLP
(B) STREET: 176 Federal Street
(C) CITY: Boston
(D) STATE: MA
15 (E) COUNTRY: USA
(F) ZIP: 02110

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
20 (C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/---
(B) FILING DATE: 13-FEB-1998
25 (C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/800,929
(B) FILING DATE: 13-FEB-1997
(C) CLASSIFICATION:

- 63 -

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Bieker-Brady, Kristina
- (B) REGISTRATION NUMBER: 39,109
- (C) REFERENCE/DOCKET NUMBER: 07891/009WO2

5 (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617-428-0200
- (B) TELEFAX: 617-428-7045
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (D) OTHER INFORMATION: Xaa at positions 2, 3, 4, 5, 6, 7, 9, 10, 11, 17, 18, 19, 20, 21, 23, 25, 30, 31, 32, 34, 35, 38, 39, 40, 41, 42, and 45 may be any amino acid. Xaa at position 8 is Glu or Asp. Xaa at positions 14 & 22 is Val or Ile.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Glu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Lys	Xaa	Cys	Met
1				5						10					15	
25	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Phe	Xaa	Pro	Cys	Gly	His	Xaa	Xaa	Xaa
				20						25					30	
	Cys	Xaa	Xaa	Cys	Ala	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Pro	Xaa	Cys		
				35						40					45	

(2) INFORMATION FOR SEQ ID NO:2:

- 64 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa at positions 1, 2, 3,

6, 9, 10, 14, 15, 18, 19, 20, 21, 24, 30, 32, 33, 35, 37, 40,
 42, 43, 44, 45, 46, 47, 49, 50, 51, 53, 54, 55, 56, 57, 59, 60,
 61, 62, 64 and 66 may be any amino acid. Xaa at positions 13, 16 and
 17 may be any amino acid or may be absent.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

15 Xaa Xaa Xaa Arg Leu Xaa Thr Phe Xaa Xaa Trp Pro Xaa Xaa Xaa Xaa
 1 5 10 15
 Xaa Xaa Xaa Xaa Xaa Leu Ala Xaa Ala Gly Phe Tyr Tyr Xaa Gly Xaa
 20 25 30
 Xaa Asp Xaa Val Xaa Cys Phe Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Trp
 20 35 40 45
 Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Xaa Pro Xaa
 50 55 60
 Cys Xaa Phe Val
 65

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5232 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 65 -

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

	GAAAAGGTGG ACAAGTCCTA TTTTCAAGAG AAGATGACTT TTAACAGTTT TGAAGGATCT	60
	AAAACCTTGTG TACCTGCAGA CATCAATAAG GAAGAAGAAT TTGTAGAAGA GTTTAATAGA	120
5	TTAAAACTT TTGCTAATTT TCCAAGTGGT AGTCCTGTTT CAGCATCAAC ACTGGCACGA	180
	GCAGGGTTTC TTTATACTGG TGAAGGAGAT ACCGTGCGGT GCTTTAGTTG TCATGCAGCT	240
	GTAGATAGAT GGCAATATGG AGACTCAGCA GTTGGAAGAC ACAGGAAAGT ATCCCCAAAT	300
	TGCAGATTTA TCAACGGCTT TTATCTTGAA AATAGTGCCA CGCAGTCTAC AAATTCTGGT	360
	ATCCAGAATG GTCAGTACAA AGTTGAAAAC TATCTGGGAA GCAGAGATCA TTTTGCCTTA	420
10	GACAGGCCAT CTGAGACACA TGCAGACTAT CTTTTGAGAA CTGGGCAGGT TGATAGATATA	480
	TCAGACACCA TATACCCGAC GAACCCCTGCG ATGTATAGTG AAGAAGCTAG ATTAAAGTCC	540
	TTTCAGAACT GGCACAGACTA TGCTCACCTA ACCCCAAGAG AGTTAGCAAG TGCTGGACTC	600
	TACTACACAG GTATTGGTGA CCAAGTGCAAG TGCTTTTGTT GTGGTGGAAA ACTGAAAAAT	660
	TGGGAACCTT GTGATCGTGC CTGGTCAGAA CACAGGCGAC ACTTTCCTAA TGGCTTCTTT	720
15	GTTTTGGGCC GGAATCTTAA TATTCGAAGT GAATCTGATG CTGTGAGTTC TGATAGGAAT	780
	TTCCCAAATT CAACAAATCT TCCAAGAAAT CCATCCATGG CAGATTATGA AGCACGGATC	840
	TTTACTTTTG GGACATGGAT ATACTCAGTT AACAAGGAGC AGCTTCCAAG AGCTGGATTT	900
	TATGCTTTAG GTGAAGGTGA TAAAGTAAAG TGCTTTCCTT GTGGAGGAGG GCTAACTGAT	960
	TGGAAGCCCA GTGAAGAGCC TTGGGAACAA CATGCTAAAT GGTATCCAGG GTGCAAATAT	1020
20	CTGTTAGAAC AGAAGGGACA AGAATATATA AACAATATTC ATTTAACTCA TTCACTTGAG	1080
	GAGTGTCTGG TAAGAACTAC TGAGAAAACA CCATCACTAA CTAGAAGAAT TGATGATACC	1140
	ATCTTCCAAA ATCCTATGGT ACAAGAAGCT ATACGAATGG GGTTCAGTTT CAAGGACATT	1200
	AAGAAAATAA TGGAGGAAAA AATTGAGATA TCTGGGAGCA ACTATAAATC ACTTGAGGTT	1260
	CTGGTTGCAG ATCTAGTGAA TGCTCAGAAA GACACTATGC AAGATGAGTC AAGTCAGACT	1320
25	TCATTACAGA AAGAGATTAG TACTGAAGAG CAGCTAAGGC GCCTGCAAGA GGAGAAGCTT	1380
	TGCAAAATCT GATATGGATAG AAATATTGCT ATCGTTTTTG TTCCTTGTGG ACATCTAGTC	1440
	ACTTGTA AAC AATGTGCTGA AGCAGTTGAC AAGTGTCCCA TGTGCTACAC AGTCATTACT	1500
	TTCAAGCAAA AAATTTTAT GTCTTAATCT AACTCTATAG TAGGCATGTT ATGTTGTTCT	1560
	TATTACCTG ATTGAATGTG TGATGTGAAC TCACTTTAAG TAATCAGGAT TGAATTCCAT	1620
30	TAGCATTGTC TACCAAGTAG GAAAAAAAT GTACATGGCA GTGTTTTAGT TGGCAATATA	1680
	ATCTTTGAAT TTCTTGATTT TTCAGGGTAT TAGCTGTATT ATCCATTTTT TTTACTGTTA	1740
	TTTAATTGAA ACCATAGACT AAGAATAAGA AGCATCATAC TATAACTGAA CACAATGTGT	1800
	ATTCATAGTA TACTGATTTA ATTTCTAAGT GTAAGTGAAT TAATCATCTG GATTTTTTAT	1860
	TCTTTTCAGA TAGGCTTAAC AAATGGAGCT TTCTGTATAT AAATGTGGAG ATTAGAGTTA	1920
35	ATCTCCCCAA TCACATAATT TGTTTTGTGT GAAAAGGAA TAAATTGTTT CATGCTGGTG	1980

- 66 -

	GAAAGATAGA GATTGTTTTT AGAGGTGGT TGTGTGTTT TAGGATTCTG TCCATTTTCT	2040
	TTTAAAGTTA TAAACACGTA CTTGTGCGAA TTATTTTTT AAAGTGATT GCCATTTTTG	2100
	AAAGCGTATT TAATGATAGA ATACTATCGA GCCAACATGT ACTGACATGG AAAGATGTCA	2160
	AAGATATGTT AAGTGTAATA TGCAAGTGGC AAAACACTAT GTATAGTCTG AGCCAGATCA	2220
5	AAGTATGTAT GTTTTAAATA TGCATAGAAC AAAAGATTTG GAAAGATATA CACCAAACTG	2280
	TTAAATGTGG TTTCTCTTCG GGGAGGGGGG GATTGGGGGA GGGGCCCCAG AGGGGTTTTA	2340
	TAGGGGCCCTT TTCACTTTCT ACTTTTTTCA TTTTGTCTG TTCGAATTTT TTATAAGTAT	2400
	GTATTACTTT TGAATCAGA ATTTTATGAA AGTATTTTGC TGATTTAAAG GCTTAGGCAT	2460
	GTTCAAACGC CTGCAAACT ACTTATCACT CAGCTTAGT TTTTCTAATC CAAGAAGGCA	2520
10	GGGCAGTTAA CCTTTTTGGT GCCAATGTGA AATGTAAATG ATTTTATGTT TTTCTGCTT	2580
	TGTGGATGAA AAATATTTCT GAGTGGTAGT TTTTGACAG GTAGACCATG TCTTATCTTG	2640
	TTTCAAAATA AGTATTTCTG ATTTTGTAAA ATGAAATATA AAATATGTCT CAGATCTTCC	2700
	AATTAATTAG TAAGGATTCA TCCTTAATCC TTGCTAGTTT AAGCCTGCCT AAGTCACTTT	2760
	ACTAAAAGAT CTTTGTTAAC TCAGTATTTT AAACATCTGT CAGCTTATGT AGGTAAAAGT	2820
15	AGAAGCATGT TTGTACACTG CTGTAGTTA TAGTGACAGC TTTCCATGTT GAGATTCTCA	2880
	TATCATCTTG TATCTTAAAG TTTCATGTA GTTTTTACCG TTAGGATGAT TAAGATGTAT	2940
	ATAGGACAAA ATGTTAAGTC TTTCTCTAC CTACATTTGT TTTCTGGCT AGTAATAGTA	3000
	GTAGATACTT CTGAAATAAA TGTTCTCTCA AGATCCTTAA AACCTCTTGG AAATTATAAA	3060
	AATATTGGCA AGAAAAGAAG AATAGTTGTT TAAATATTTT TTAATAAACA CTTGAATAAG	3120
20	AATCAGTAGG GTATAAACTA GAAGTTTAAA AATGCCTCAT AGAACGTCCA GGGTTTACAT	3180
	TACAAGATTC TCACAACAAA CCCATTGTAG AGGTGAGTAA GCCATGTTAC TACAGAGGAA	3240
	AGTTTGAGAG TAAACTGTA AAAAATTATA TTTTGTGTGT ACTTTCTAAG AGAAAGAGTA	3300
	TTGTTATGTT CTCCTAAGT CTGTTGATTA CTACTTTAAG TGATATTCAT TTAACAATT	3360
	GCAAATTTAT TTTATTTAT TAATTTTCTT TTTGAGATGG AGTCTTGCTT GTCACCCAGG	3420
25	CTGGAGTGCA GTGGAGTGAT CTCTGCTCAC TGCAACCTCC GCCTTCTGGG TTCAAGCGAT	3480
	TCTCGTGCCT CAGCTTCCTG AGTAGCTGGA ATTACAGGCA GGTGCCACCA TGCCCCACTA	3540
	ATTTTTTTTT ATTTTATAGT GAGACGGGGT TTCACCATGT TGGCCAGGCT GGTATCAAAC	3600
	TCCTGACCTC AAGAGATCCA CTCGCCTTGC CCTCCCAAAG TGCTGGGATT ACAGGCTTGA	3660
	GCCACCACGC CCGGCTAAAA CATTGCAAAT TTAAATGAGA CTTTTAAAAA TTAATAATG	3720
30	ACTGCCCTGT TTCTGTTTTA GTATGTAAAT CCTCAGTTCT TCACCTTTGC ACTGTCTGCC	3780
	ACTTAGTTTG GTTATATAGT CATTAACTTG AATTTGGTCT GTATAGTCTA GACTTTAAAT	3840
	TTAAAGTTTT CTACAAGGGG AGAAAAGTGT TAAATTTTTT AAAATATGTT TTCCAGGACA	3900
	CTTCACCTCC AAGTCAGGTA GGTAGTTCAA TCTAGTTGTT AGCCAAGGAC TCAAGGACTG	3960
	AATTGTTTTA ACATAAGGCT TTTCTGTTC TGGGAGCCGC ACTTCATTAA AATTCTTCTA	4020
35	AACTTGATAT GTTTAGAGTT AAGCAAGACT TTTTCTTTC CTCTCCATGA GTTGTGAAAT	4080
	TTAATGCACA ACGCTGATGT GGCTAACAAG TTTATTTTAA GAATTGTTTA GAAATGCTGT	4140
	TGCTTCAGGT TCTTAAATC ACTCAGCACT CCAACTTCTA ATCAAATTTT TGGAGACTTA	4200

- 67 -

ACAGCATTG TCTGTGTTG AACTATAAAA AGCACCGGAT CTTTCCATC TAATTCCGCA 4260
 AAAATTGATC ATTTGCAAAG TCAAACTAT AGCCATATCC AAATCTTTTC CCCCTCCCAA 4320
 GAGTTCTCAG TGTCTACATG TAGACTATTC CTTTTCTGTA TAAAGTTCAC TCTAGGATTT 4380
 CAAGTCACCA CTTATTTTAC ATTTTAGTCA TGCAAAGATT CAAGTAGTTT TGCAATAAGT 4440
 5 ACTTATCTTT ATTTGTAATA ATTTAGTCTG CTGATCAAAA GCATTGTCTT AATTTTGTAG 4500
 AACTGGTTTT AGCATTTACA AACTAAATC CAGTTAATTA ATTAATAGCT TTATATTGCC 4560
 TTTCCTGCTA CATTTGGTTT TTTCCCTGT CCCTTTGATT ACGGGCTAAG GTAGGGTAAG 4620
 AXXGGGTGTA GTGAGTGAT ATAATGTGAT TTGGCCCTGT GTATTATGAT ATTTTGTAT 4680
 TTTTGTGTT ATATTATTTA CATTTCAGTA GTTGTTTTTT GTGTTTCCAT TTTAGGGGAT 4740
 10 AAAATTGTG TTTTGAATA TGAATGGAGA CTACGCCCC AGCATTAGTT TCACATGATA 4800
 TACCCTTTAA ACCCGAATCA TTGTTTTATT TCCTGATTAC ACAGGTGTTG AATGGGGAAA 4860
 GGGGCTAGTA TATCAGTAGG ATATACTATG GGATGTATAT ATATCATTGC TGTAGAGAA 4920
 ATGAAATAAA ATGGGCTGG GCTCAGTGGC TCACGCCTGT AATCCCAGCA CTTGGGAGG 4980
 CTGAGGCAGG TGGATCAGG GGTGAGGAGA TCGAGACCAT CCTGGCTAAC ACGGTGAAAC 5040
 15 CCCGTCTCTA CTAATAAACA GAAATTAGC CGGGCGTGGT GGCGGGCGCC TGTAGTCCCA 5100
 GCTACTCGGG AGGCTGAGGC AGGAGAATGG TGTGAACCG GGAGGCAGAG CTTGCAGTGA 5160
 GCCGAGATCT CGCCACTGCA CTCCAGCCTG GGCAACAGAG CAAGACTCTG TCTCAAAAAA 5220
 AAAAAAAAAA AG 5232

(2) INFORMATION FOR SEQ ID NO:4:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 497 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Phe Asn Ser Phe Glu Gly Ser Lys Thr Cys Val Pro Ala Asp
 1 5 10 15
 Ile Asn Lys Glu Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
 20 25 30
 Phe Ala Asn Phe Pro Ser Gly Ser Pro Val Ser Ala Ser Thr Leu Ala
 35 40 45

- 68 -

Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Arg Cys Phe
 50 55 60
 Ser Cys His Ala Ala Val Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
 65 70 75 80
 5 Gly Arg His Arg Lys Val Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
 85 90 95
 Tyr Leu Glu Asn Ser Ala Thr Gln Ser Thr Asn Ser Gly Ile Gln Asn
 100 105 110
 Gly Gln Tyr Lys Val Glu Asn Tyr Leu Gly Ser Arg Asp His Phe Ala
 10 115 120 125
 Leu Asp Arg Pro Ser Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
 130 135 140
 Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
 145 150 155 160
 15 Tyr Cys Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
 165 170 175
 Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
 180 185 190
 Gly Ile Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
 20 195 200 205
 Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
 210 215 220
 Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Leu Asn Ile Arg Ser Glu
 225 230 235 240
 25 Ser Asp Ala Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Leu
 245 250 255
 Pro Arg Asn Pro Ser Met Ala Asp Tyr Glu Ala Arg Ile Phe Thr Phe
 260 265 270
 Gly Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly
 30 275 280 285
 Phe Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly
 290 295 300
 Gly Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Glu Gln His
 305 310 315 320
 35 Ala Lys Trp Tyr Pro Gly Cys Lys Tyr Leu Leu Glu Gln Lys Gly Gln
 325 330 335
 Glu Tyr Ile Asn Asn Ile His Leu Thr His Ser Leu Glu Glu Cys Leu

- 69 -

340 345 350
 Val Arg Thr Thr Glu Lys Thr Pro Ser Leu Thr Arg Arg Ile Asp Asp
 355 360 365
 Thr Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe
 5 370 375 380
 Ser Phe Lys Asp Ile Lys Lys Ile Met Glu Glu Lys Ile Gln Ile Ser
 385 390 395 400
 Gly Ser Asn Tyr Lys Ser Leu Glu Val Leu Val Ala Asp Leu Val Asn
 405 410 415
 10 Ala Gln Lys Asp Ser Met Gln Asp Glu Ser Ser Gln Thr Ser Leu Gln
 420 425 430
 Lys Glu Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys
 435 440 445
 Leu Cys Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Val Pro
 15 450 455 460
 Cys Gly His Leu Val Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys
 465 470 475 480
 Cys Pro Met Cys Tyr Thr Val Ile Thr Phe Lys Gln Lys Ile Phe Met
 485 490 495
 20 Ser

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 6669 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30 TTGCTCTGTC ACCCAGTTTG GAGTGCAGTT ATGCAGTCTC AACTGCAAG CTCTGCCTCA 60
 TGGGCTCAAG TGAACCTCCT GCCTCAGCCT CTCAAGTAGC TGGGACCACA GGCAGGTGCC 120
 ACCATGTCTG GCTAATTTT GAGTTTCTTT GTAGAGATGG TGTTTTGCCA AGTCACCCAC 180

- 70 -

	TTTGAGGCTG	GTCTCAAACA	CCTGGGCTCA	AGCAATCCAT	CTACCTCAGC	CTCCCAAAGT	240
	GCTGGGATTA	CAGGAGTGAG	CCATGGCATG	AGGCCTTGTG	GUGTGTCTCT	TTTAAATGAA	300
	AGCATACTCT	GTTTACGTAT	TTGATATGAA	CGAATATCCT	TCCTTTCCAC	AAAGACAAAA	360
	ATTATCCTAT	TTTTCTCAAA	ACATATGTCC	TTTTCTCTA	CTTTTCATTT	TTGTTACTTT	420
5	TGATGGACAC	ATGTGTTACA	TTGATTTTAC	TTTCTCATAA	TTCTGCTGTA	AGAAAAACAA	480
	TAGTGCCAGT	TCAATGACAA	ATAGCAACAG	TCTGTTATTG	CTAGACTGTT	ACTGTTAGTG	540
	GAGACTACCA	GAACAGTCAG	TCCCAGTGTC	AGGGAATCAA	AGAGAACATG	TTCCCTCTCT	600
	AAAGGGCACA	GCTGCTGCTC	AGCTTTAGCT	GATTGCTGCC	CTGCAGGACT	ATAGGCCCCAG	660
	TGTTGCTAGA	TCTTTTGATG	TTTCAAGAGA	AGCTTGAAT	CTAGAATGTG	ATGGGAAGTC	720
10	TCTTACATTT	AAACATGTTG	GCAATTAATG	GTAAGATTTA	AAAATACTGT	GGTCCAAGAA	780
	AAAAATGGAT	TTGGAAACTG	GATTAAATTC	AAATGAGGCA	TGCAGATTAA	TCTACAGCAT	840
	GGTACAATGT	GAATTTTCTG	GTTTCTTTAA	TTGCACTGTA	ATTAGSTAAG	ATGTTAGCTT	900
	TGGGGAAGCT	AAGTGCAGAG	TATGCAGAAA	CTATTATTTT	TGTAAGTTTT	CTCTAAGTAT	960
	AAATAAATTT	CAAAAATAAA	ATAAAAACCT	AGTAAAGAAC	TATAATGCAA	TTCTATGTAA	1020
15	CCCAAAACATA	ATATGTCTTC	CAGTTTGAAA	CCTCTGGGTT	TTATTTTATT	TTATTTTATT	1080
	TTTGAGACAG	AGTCTTGCTG	TGTCACCCAG	GCTGGAGTGT	AGTGGCACTA	TTTCGGCCCCA	1140
	CTGCAACCTC	CACCTCCCAG	GCTCAAATGA	TTCTCTGCCC	TCAGCCTCCG	GAGTAGCTGG	1200
	GATTACAGGC	GCGTACCACC	ACACCCAGCT	AATTTTTGTA	TTTTTAGTAG	AGATGGGGTT	1260
	TCACCATTTT	GGCCAGGCTG	GTTTTGAACT	CCTGACCTCA	AGTGATCCAC	TTGTCTTGCC	1320
20	CTCCCAAAAT	GCTGGGATTA	CAGGCGTGAG	CCACTGCACC	AGGCAGAGGC	CTCTGTTTTT	1380
	TATCTCTTTT	TGGCCTCTAC	AGTGCCTAGT	AAAGCACCTG	ATACATGGTA	AACGATCAGT	1440
	AATTACTAGT	ACTCTATTTT	GGAGAAAATG	ATTTTTTAAA	AAGTCATTGT	GTTCCATCCA	1500
	TGAGTCGTTT	GAGTTTTTAA	ACTGTCTTTT	TGTTTGTTTT	TGAACAGGTT	TACAAAGGAG	1560
	GAAAACGACT	TCTTCTAGAT	TTTTTTTTCA	GTTTCTCTTA	TAAATCAAAA	CATCTCAAAA	1620
25	TGGAGACCTA	AAATCCTTAA	AGGGACTTAG	TCTAATCTCG	GGAGGTAGTT	TTGTGCATGG	1680
	GTAACAAAT	TAAGTATTAA	CTGGTGTTTT	ACTATCCALA	GAATGCTAAT	TTTATAAACA	1740
	TGATCGAGTT	ATATAAGGTA	TACCATAATG	AGTTTGATTT	TGAATTTGAT	TTGTGGAAAT	1800
	AAAGGAAAAG	TGATTCTAGC	TGGGGCATAT	TGTTAAAGCA	TTTTTTTCAG	AGTTGGCCAG	1860
	GCAGTCTCCT	ACTGGCACAT	TCTCCCATTA	TGTAGAATAG	AAATAGTACC	TGTGTTTGGG	1920
30	AAAGATTTTA	AAATGAGTGA	CAGTTATTTG	GAACAAAGAG	CTAATAATCA	ATCCACTGCA	1980
	AATTAAAGAA	ACATGCAGAT	GAAAGTTTTG	ACACATTAAA	ATACTTCTAC	AGTGACAAAG	2040
	AAAAATCAAG	AACAAAGCTT	TTTGATATGT	GCAACAAATT	TAGAGGAAGT	AAAAAGATAA	2100
	ATGTGATGAT	TGGTCAAGAA	ATTATCCAGT	TATTTACAAG	GCCACTGATA	TTTTAAACGT	2160
	CCAAAAGTTT	GTTTAAATGG	GCTGTTACCG	CTGAGAATGA	TCAGGATGAG	AATGATGGTT	2220
35	GAAGGTTACA	TTTtagGAAA	TGAAGAACT	TAGAAAATTA	ATATAAGAC	AGTGATGAAT	2280
	ACAAAGAAGA	TTTTTATAAC	AATGTGTAAG	ATTTTGGCC	AGGGAAAGGA	ATATTGAAGT	2340
	TAGATACAAT	TACTTACCTT	TGAGGGAAAT	AATTGTTGGT	AATGAGATGT	GATGTTTCTC	2400

- 71 -

	CTGCCACCTG GAAACAAAGC ATTGAACTCT GCAGTTGAAA AGCCCAACGT CTGTGAGATC	2460
	CAGGAAACCA TGCTTGCAA CCCTGGTAA AAAAAAAAAA AAAAAAAAAA AAAGCCACAG	2520
	TGACTTGCTT ATTGGTCATT GCTAGTATTA TCGACTCAGA ACCTCTTIAC TAATGGCTAG	2580
	TAAATCATAA TTGAGAAATT CTGAATTTTG ACAAGGTCTC TGCTGTTCAA ATGSTAAATT	2640
5	TATTATTTTT TTTGTCATGA TAAATCTGCG TTCAAGGTAT GCTATCCATG AAATAATTTT	2700
	TGACCAAAAC TAAATTGATG CAATTTGATT ATCCATCTTA GCCTACAGAT GGCATCTGGT	2760
	AACTTTTGAC TGTTTTAAAA AATAAATCCA CTATCAGAGT AGATTTGATG TTGGCTTCAG	2820
	AAACATTAG AAAACAAAA GTTCAAAAAT GTTTTCAGGA GGTGATAAGT TGAATAACTC	2880
	TACAATGTTA GTTCTTTGAG GGGGACAAAA AATTTAAAT CTTTGAAAGG TCTTATTTTA	2940
10	CAGCCATATC TAAATTATCT TAAGAAAATT TTAAACAAAG GGAATGAAAT ATATATCATG	3000
	ATTCTGTTTT TCCAAAAGTA ACCTGAATAT AGCAATGAAG TTCAGTTTTG TTATTGGTAG	3060
	TTTGGGCAGA GTCTCTTTTT GCAGCACCTG TTGCTACCA TAATTACAGA GGACATTTCC	3120
	ATGTTCTAGC CAAGTATACT ATTAGAATAA AAAAAGTTAA CATTGAGTTG CTTCAACAGC	3180
	ATGAACTGA GTCCAAAAGA CCAATGAAC AAACACATTA ATCTCTGATT ATTTATTTTA	3240
15	AATAGAATAT TTAATTGTGT AAGATCTAAT AGTATCATT TACTTAAGCA ATCATATTCC	3300
	TGATCATCTA TGGGAAATAA CTATTATTTA ATTAATATTG AAACCACGTT TTAAGATGTG	3360
	TTAGCCAGTC CTGTTACTAG TAAATCTCTT TACTTGAGA GAAATTTAG ATTGTTTTGT	3420
	TCTCCTTATT AGAAGGATTG TAGAAAGAAA AAAATGACTA ATGGGAGAAA AATTGGGGAT	3480
	ATATCATATT TCACTGAATT CAAAATGTCT TCAGTTGTAA ATCTTACCAT TATTTTACGT	3540
20	ACCTCTAAGA AATAAAGTG CTTCTAATTA AAATATGATG TCATTAATTA TGAAATACTT	3600
	CTTGATAACA GAAGTTTTAA AATAGCCATC TTAGAATCAG TGAAATATGG TAATGTATTA	3660
	TTTTCTCCT TTGAGTNAGG TCTTGTGCTT TTTNTTCCTG GCCACTAAAT NTCACCATNT	3720
	CCANAAGCA AANTAAACCT ATTCTGAATA TTTTGTCTGT GAAACACTTG NCAGCAGAGC	3780
	TTTCCCNCCA TGNNAGAAGC TTCATGAGTC ACACATTACA TCTTTGGGTT GATTGAATGC	3840
25	CACTGAAACA TTTCTAGTAG CCTGGAGNAG TTGACCTACC TGTGGAGATG CCTGCCATTA	3900
	AATGGCATCC TGATGGCTTA ATACACATCA CTCTCTGTG NAGGGTTTTA ATTTTCAACA	3960
	CAGCTTACTC TGATGATCA TGTTTACATT GTATGTATAA AGATTATACN AAGGTGCAAT	4020
	TGTGTATTTT TTCCTTAAAA TGTATCAGTA TAGGATTTAG AATCTCCATG TTGAACTCT	4080
	AAATGCATAG AAATAAAAAA AATAAAAAAT TTTTCATTTT GGCTTTTCAG CTTAGTATTA	4140
30	AAACTGATAA AAGCAAAGCC ATGCACAAAA CTACCTCCCT AGAGAAAGGC TAGTCCCTTT	4200
	TCTTCCCAT TCATTTTATT ATGAACATAG TAGAAAACAG CATATTCTTA TCAAATTTGA	4260
	TGAAAAGCGC CAACACGTTT GAACTGAAAT ACGACTTGTC ATGTGAACTG TACCGAATGT	4320
	CTACGTATTC CACTTTTCCT GCTGGGGTTC CTGTCTCAGA AAGGAGTCTT GCTCGTGCTG	4380
	GTTTCTATTA CACTGGTGTG AATGACAAGG TCAAATGCTT CTGTTGTGTC CTGATGCTGG	4440
35	ATAACTGGAA AAGAGGAGAC AGTCCTACTG AAAAGCATAA AAAGTTGTAT CCTAGCTGCA	4500
	GATTTCGTTCA GAGTCTAAAT TCCGTTAACA ACTTGAAGC TACCTCTCAG CCTACTTTTC	4560
	CTTCTTCAGT AACACATTCC ACACACTCAT TACTTCCGGG TACAGAAAAC AGTGGATATT	4620

- 72 -

	TCCGTGGCTT	TTATTCAAAC	TCTCCATCAA	ATCCTTTAAA	CTCCAGAGCA	AATCAAGAAT	4680
	TTTCTGCTT	GATGAGAAAT	TCTTACCTTT	GTCCAAATGA	TAACGAAAAT	CCCAGATTAC	4740
	TTACTTTTCA	GACATGGCCA	TTGACTTTTC	TCTGCCCCAC	AGATCTGSCA	CGAGCAGGCT	4800
	TTTACTACAT	AGGACCTGGA	GACAGAGTGG	CTTGCTTTTC	CTGTGGTGGG	AAATTGAGCA	4860
5	ATTGGGAACC	GAAGGATAAT	GCTATGTGAG	AACACCTGAG	ACATTTTCCC	AAATGCCCAT	4920
	TTATAGAAAA	TCAGCTTCAA	GACACTTCAA	GATACACAGT	TTCTAATCTG	AGCATGCAGA	4980
	CACATGCAGC	CCGCTTTAAA	ACATTCTTTA	ACTGCCCCTC	TAGTGTCTTA	GTTAATCTTG	5040
	AGCAGCTTTC	AAGTGGGGGT	TTTTATTATG	TGGSTAACAG	TCATGATGTC	AAATGCTTTT	5100
	GCTGTGATGG	TGSACTCAGG	TGTTGGGAAT	CTGGAGATGA	TCCATGGGTT	CAACATGCCA	5160
10	ACTGCTTTCC	AAGGTGTGAG	TACTTGATAA	GAATTAAAGG	ACAGGAGTTC	ATCCGTCAAG	5220
	TTCAAACCCAG	TTACCCCTCAT	CTACTTGAAC	AGCTGCTATT	CACATCAGAC	AGCCCAGGAG	5280
	ATGAATAATGC	AGAGTCTCA	ATTATCCATT	TTGAACCTGG	AGAAGACCAT	TCAGAAGATG	5340
	CAATCATGAT	CAATCTCTCT	GTGATTAATG	CTGCGCTGCA	AATGGGCTTT	AGTAGAAGCC	5400
	TGCTAAAACA	GACACTTCAG	AGAAAATCT	TAGCACTGCG	AGAGAATTAT	AGACTAGTCA	5460
15	ATGATCTTGT	GTTAGACTTA	CTCAATGCGG	AAGATGAAT	AATGGAGAGG	GAGAGAGGAA	5520
	GAGCAACTGA	GGAAAAAGAA	TCAATATATT	TATATTTAAT	CGGGAAGAAT	AGAATGGCAC	5580
	TTTTTCAACA	TTTCACTGCT	GTATTTGAAA	TTCTGATAG	TCTACTAATC	CCCGGAATTA	5640
	TTATGAACA	AGAACATGAT	GTATTTAAG	AGAAACACAA	GAGCTTTTAA	CAAGCAAGAG	5700
	AAGTATTTGA	TACGATTTTA	GTAAAAGGAA	ATATTGACAG	CAGTGTATTG	AGAATCTCTC	5760
20	TGCAAGAGAG	TGAAGCTGTG	TTATATGAGG	ATTATTTTGT	GGACAGGAG	ATAAATATA	5820
	TTCCACACAG	AGATGTTTCA	GATCTACCCG	TGGAGAGACA	ATTGCGGAGA	CTACAAGAG	5880
	AAAGAACATG	TAAATGTGT	ATGGACAAAG	AAGTCTCCAT	AGTGTTTATT	CCTGTGCTC	5940
	ATCTAGTAGT	ATGCAAGAT	TGTCTCTCTT	CTTAAGAAA	GTCTCTATT	TGTAGGAGTA	6000
	CAATCAAGGG	TACAGTTTGT	ACATTTCTTT	CATGAGAGAG	AGCAGAGACA	TGCTCTAAG	6060
25	TTTAGAATTA	ATTIATTTAA	TGTATTATAA	CTTTAACTTT	TATCTAATT	TGGTTCTCT	6120
	AAATATTTTA	TTTATTTACA	ACTCAAAATA	CATTGTTTTG	TCTAACATAT	TTATATATGT	6180
	ATCTAAACCA	TATGAACATA	TATTTTCTAG	AAACTAAGAG	AATGATAGGC	TTTGTCTCT	6240
	ATGAACGAAA	AAGAGGTAGC	ACTACAGACA	CAATATTCAA	TCAAAATTTT	AGCATTATTC	6300
	AAATTGTAA	TGAATTAATA	CTTAAGATAT	TTGATTTAAG	CTTTAAGAA	TTTAAATATT	6360
30	TTGGCATTGT	ACTAATACCG	GGAACATGAA	GCCAGCTCTG	GTGCTATCTG	CCTGTAGTCC	6420
	CAGCTCTAGG	CAAGAGAAAT	ATTGAGCTCT	AGGACTTTTA	ATGATCTCTG	GCCAGGATAC	6480
	TGAGACCTTG	CCTTTAAAAA	CAACAGAGAG	AAAAACAAAA	CAACAGGGAG	ACATTTCTCT	6540
	GTCTTTCTTG	ATCACTGTCC	TATACATCGA	AGGTGTCCAT	ATATTTGAA	TCACATTTTA	6600
	GGGACATGCT	GTTTTATATA	AGAATTCTCT	GAGAAAAAAT	TTAATAAAGC	AACCAAAAAA	6660
35	AAAAAAAA						6669

© INFORMATION FOR SEQ ID NO:6:

- 73 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 604 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Asn Ile Val Glu Asn Ser Ile Phe Leu Ser Asn Leu Met Lys Ser
  1             5             10             15
10 Ala Asn Thr Phe Glu Leu Lys Tyr Asp Leu Ser Cys Glu Leu Tyr Arg
    20             25             30
Met Ser Thr Tyr Ser Thr Phe Thr Ala Thr Glu Asp Leu Ser Glu Arg
    35             40             45
Ser Leu Ala Arg Ala Gly Phe Lys Tyr Thr Gly Thr Asn Asp Lys Val
15 50             55             60
Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Arg Gly Asp
    65             70             75             80
Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser Cys Arg Phe Val
    85             90             95
20 Gln Ser Leu Asn Ser Val Asn Asn Leu Glu Ala Thr Ser Gln Pro Thr
    100            105            110
Phe Pro Ser Ser Val Thr His Ser Thr His Ser Leu Leu Pro Gly Thr
    115            120            125
Glu Asn Ser Gly Tyr Phe Arg Gly Ser Tyr Ser Asn Ser Pro Ser Asn
25 130            135            140
Pro Val Asn Ser Arg Ala Asn Gln Glu Phe Ser Ala Leu Met Arg Ser
    145            150            155            160
Ser Tyr Pro Cys Pro Met Asn Asn Glu Asn Ala Arg Leu Leu Thr Phe
    165            170            175
30 Gln Thr Trp Pro Leu Thr Phe Leu Ser Pro Thr Asp Leu Ala Arg Ala
    180            185            190
Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
    195            200            205
Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asn Ala Met Ser Glu

```

- 74 -

	310	315	320
	His Leu Arg His Phe Pro Lys Lys Pro Phe Ile Gln Asn Gln Leu Gln		
	325	330	335
	Asp Thr Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala		
5	245	250	255
	Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu Val Asn		
	260	265	270
	Pro Gln Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn Ser Asp		
	275	280	285
10	Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser		
	290	295	300
	Gly Asp Asp Pro Trp Val Ile His Ala Lys Trp Phe Pro Arg Cys Glu		
	305	310	315
	Tyr Leu Ile Arg Ile Lys Gly Gln Gln Phe Ile Arg Gln Val Gln Ala		
15	325	330	335
	Ser Tyr Pro His Leu Leu Gln Gln Leu Leu Ser Thr Ser Asp Ser Pro		
	340	345	350
	Gly Asp Glu Asn Ala Gln Ser Ser Ile Ile His Leu Glu Pro Gly Glu		
	355	360	365
20	Asp His Ser Glu Asp Ala Ile Met Met Asn Thr Pro Val Ile Asn Ala		
	370	375	380
	Ala Val Glu Met Gly Phe Ser Arg Ser Leu Val Lys Gln Thr Val Gln		
	385	390	395
	Arg Lys Ile Leu Ala Thr Gly Glu Asn Tyr Arg Leu Val Asn Asp Leu		
25	405	410	415
	Val Leu Asp Leu Leu Asn Ala Glu Asp Glu Ile Arg Glu Glu Glu Arg		
	420	425	430
	Glu Arg Ala Thr Glu Glu Lys Glu Ser Asn Asp Leu Leu Leu Ile Arg		
	435	440	445
30	Lys Asn Arg Met Ala Leu Phe Gln His Leu Thr Tyr Val Ile Pro Ile		
	450	455	460
	Leu Asp Ser Leu Leu Thr Ala Gly Ile Ile Asn Glu Gln Glu His Asp		
	465	470	475
	Val Ile Lys Gln Lys Thr Gln Thr Ser Leu Gln Ala Arg Glu Leu Ile		
35	485	490	495
	Asp Thr Ile Leu Val Lys Gly Asn Ile Ala Ala Thr Val Phe Arg Asn		
	500	505	510

- 75 -

Ser Leu Gln Glu Ala Glu Ala Val Leu Tyr Glu His Leu Phe Val Gln
 515 520 525
 Gln Asp Ile Lys Tyr Ile Pro Thr Glu Asp Val Ser Asp Leu Pro Val
 530 535 540
 5 Glu Gln Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys
 545 550 555 560
 Met Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val
 565 570 575
 Val Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg
 580 585 590
 10 Ser Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
 595 600

D. INFORMATION FOR SEQ ID NO:10:

SEQUENCE CHARACTERISTICS

- 15 A. LENGTH: 671 base pairs
 B. TYPE: nucleic acid
 C. STRANDEDNESS: single
 D. TOPOLOGY: linear

E. MOLECULE TYPE: cDNA

- 20 F. SEQUENCE DESCRIPTION: SEQ ID NO:10:

25 GAGCGTCGCG GCTGATCCGA GCGGAGCGGG CGGTATCTCC TTCTCGGCGC CGTGATTCC 60
 CGGCTCTGCG GAGGCTCTTA GCGAGCGGCG CAGCTCCGCT GTTCTCTGCG CCGCGACTCC 120
 GATTACAGAG CCGAGAGGAT CCGCTATCTT CTATTTTCTG CCGCTGCACT AATAAATCCC 180
 ATTATCGAGA TCTCGAAAGT TTATAAAGCG ATATACTTTC AATTCTATCG AGTGTAACTT 240
 TCTGATGAAA TTATATCTTT AAAAGATTGA AGAGTTTTCG GAAAGAAAGG TAGTAGAGTT 300
 GATTACTGAT ACTTATCTCT AAGCACTACT TTTTGGGTAG TACAATATTT TGTTAGGCGT 360
 TTCIGATAAC ACTAGAAAGG ACAAGTTTTC TTTTGTGATA AATTGATTAA TGTTCACAGC 420
 ATGACTGATA ATTATAGCTG AATAGTCCTT AAATGATCAA CAGGTTATTT ACTTTTAAAA 480
 TCCAGTGTA AAGTGTCTCT GTGGAAATTT TAGGCTTAAC TAAGTTTATG GAGAAATATC 540
 30 CTCAGTTTGA TCAAGAATAA TAGTGTGATA CAAAGTTAGG AAGAAAGTTA ACATGATGCT 600
 GCAGGAAATG GAAACAAATA CAAATGATAT TTAACAAAGA TAGAGTTTAC ACTTTTGGAA 660
 CTTTAAGCCA AATTGATTTG ACATCAAGCA CTATACAGG CAGAGTTTCA ACAAAGCTTC 720

- 76 -

	TGGGTATTGA	CTTCCCCCAA	AAGTTGTCAG	CTCAASTAAT	TTAGCCCACT	TAAGTAATA	780
	CTATGATGAT	AAGCTGTGTG	AAGTTAGCTT	TTAAATAGTG	TGACCATATG	AAGGTTTTAA	840
	TTACTTTTGT	TTATTGGAAT	AAATGAGAT	TTTTTGGGTI	GTGATGTTAA	AGTGCTTATA	900
	GCGAAGAAG	CCTGCATATA	ATTTTTTACC	TTGTGGCATA	ATCAGTAATT	GCTCTGTTAT	960
5	TCAGGCTTCA	TAGCTTGTAA	CCAAATATAA	ATAAAAGGCA	TAATTTAGGT	ATTCTATAGT	1020
	TGCTTAGAAT	TTTGTTAATA	TAAATCTCTG	TGAAAAATCA	AGGAGTTTTA	ATAITTTTCA	1080
	AAGTGCATCC	ACCTTTTCAG	GCTTTAAGTT	AGTATTAACT	CAAGATTATG	AACAATAGC	1140
	ACTTAGGTTA	CCTGAAAGAG	TTACTACAAC	CCCAAGAGT	TGCTTTCTAA	GTAGTATCTT	1200
	GCTAATTCAG	AGAGATACTG	ATCCTACCTG	AATATAAACT	CAGATAAATC	CAGTAAAGAA	1260
10	AGTCTAGTAA	ATTCTACATA	AGAGTCTATC	ATTGATTTCT	TTTTGTGGTA	AAAATCTTAG	1320
	TTGATGTGAA	GAAATTTGAT	GTGAATGTTT	TAGCTATCAA	AGAGTACTGT	CACCTACTCA	1380
	TGCACAAAAC	TGCTTCCCAA	AGACTTTTTC	CAGGTCCCTG	GTATGAAAAC	ATTAAAGATA	1440
	TAATGGAABA	TAGCAGGATC	TTGTGAGATT	CGACAAACAT	CAACAAAATA	AAGATGAAGT	1500
	ATGACTTTTC	GTGTGAGCTT	TGAGGAATTT	GTAGATATTC	AAGTTTCTCT	CCCGGGGTGC	1560
15	GTGTCTCAGA	AAGGACTGTT	TTTGTGCTG	TTTTTTATTA	TATGCTGTGT	AATGACAAGG	1620
	TCAAAATGTT	CTGTGTGTGC	CTGATGTTAA	ATTAATGAAA	ATTAGGAGAT	AGTCTATTTC	1680
	AAAACATATA	AAGGATATAT	CTAGCTGTAA	CTTTTATTTA	GAATGTGCTT	TCAGCTAGTC	1740
	TGGGATCCAG	CTGTAAAGAT	AGTTCTGCAA	TGAGAAACAG	TTTTGACAT	TGATTTATCT	1800
	CCAGCTTGGA	ACATAGTACC	TTTTCAGTGT	GTCTTACTTC	CAGCTTTTCT	CCAAACCCCT	1860
20	TTAATTTCTAG	AGCAGTTGAA	GAGTCTGTTT	CATGAGGAT	TAAACCCCTAG	AGTTATGCAA	1920
	TGACTACTGA	AGAAGGCGAG	TTTCTTACCT	AGCATATGTC	GGCATTAAGT	TTTTGTGCA	1980
	CATCAGAATT	GCGAAGAGCT	GCTTTTTATT	ATATAGGACC	TGGAGATAGG	GTAGCCTGCT	2040
	TTCCCTGTGG	TGGGAAGCTC	AGTAAGTGGT	AACCAAGGAA	TGATGCTATG	TCAGAACACC	2100
	GGAGGCGATT	TCCCAACTGT	CGATTTTGTG	AAAATTTCTT	AGAAACTCTG	AGGTTTAGCA	2160
25	TTTCAATCT	GAGCATGCCAG	AGCATGTCAG	CTGAGTGGAG	AACATTTATG	TACTGGCCAT	2220
	CTAGTGTTC	AGTTGAGCCT	GAGCAGTTTG	CAAGTCTCTG	TTTTTATTAT	GTGGGTCCCA	2280
	ATGATGATGT	CAATGCTTTT	TCTTGTGATG	GTGGCTTGAG	GTGTGGGAAA	TCTGGAGATG	2340
	ATCCATGGGT	AGAAGATGCC	AAGTGTGTTT	CAAGTGTGTA	TTTCTTGATA	CGAATGAAG	2400
	GCCAGAGATT	TCTTATGAG	ATTCAAGCTA	CATATCTTTA	TTTTCTTAAA	CAGCTGTGCT	2460
30	CAACTTCAGA	TACCACTGGA	GAAGAAAATG	GTGAGGAAAT	AATTAATTAT	TTTGGACCTG	2520
	GAGAAAGTTC	TTGAGAAGAT	GTGTTTATTA	TGAATACATG	TTTGTTTAAA	TCTGCTTGG	2580
	AAATGGGCTT	TAATAGAGAG	CTGTGGAAC	AAAGCTTGA	AAGTAAATG	CTGACAACTG	2640
	GAGAGACTA	TAAACAGTTT	AATCATATTC	TGTCAGCACT	TCTTAATGCT	GAAGATGAAA	2700
	AAAGAGAAGA	GGAGAAGGAA	AAACAGCTG	AAGAAATGGC	ATCAGATGAT	TTGTCATTAA	2760
35	TTCCGAAGAA	CAGATGGCT	CTCTTTCAC	AATTGACATG	TGTGCTTCTT	ATCCTGGATA	2820
	ATCTTTTAAA	GCCCAATGTA	ATTAAATAAC	AGCAACATGA	TATTATTANA	CAAAAACAC	2880
	AGATACCTTT	ACAAGGAGAA	GAAGTCTTTC	GTAGTATTTT	GTGAAAGGA	AATGCTGCGG	2940

- 77 -

CCACATCTCT CAAAACTGT CTAAAGAAA TTGACTCTAC ATTGTATAAG AACTTATTTG 3000
 TGGATAAGAA TATGAAGTAT ATCCACACAG AAGATGTTTC AGGTCTCTCA CTGGAAGAAC 3060
 AATTGAGGAG GTTGCAAGAA GAACGAACCT GTAAAGTCTG TATGGACAAA GAAGTTTCTG 3120
 TTGTATTCTAT TCCTTGTTGGT CATCTGCTAG TATGCCAGGA ATGTGCCCTT TCTCTAAGAA 3180
 5 AAIGCCCTAT TTGCGGGGT ATATCAAGG GTACTGTTCG TACATTCTCT TCTTAAAGAA 3240
 AAATAGTCTA TATTTTAACC TGCATAAAA GGTCTTTAAA ATATTGTGTA ACACTTGAAG 3300
 CCATCTAAG TAAAAAGGGA ATTATGAGTT ITTCAATTAG TAACATTCAT GTCTAGTCT 3360
 GCTTTGGTAC TAATAATCTT GTTCTGAAA AGATGGTATC ATATATTAA TCTTAATCTG 3420
 TTTATTTACA AGGGAAGATT TATGTTTGT GAACTATATT AATATGATG TGTACCTAAG 3480
 10 GGAGTAGTGT CACTGCTTGT TATGCATCAT TTCAGGAGTT ACTGGATTTC TTGTTCTTTC 3540
 AGAAAGCTTT GAATACTAAA TTATAGTCTA GAAAGAACT GAAACCAGG AACTCTGGAG 3600
 TTCATCAGAG TTATGCTCCG GAATTGCTTT TGGTGGTTTT CACTTGTCTT TTAATAAAG 3660
 GATTTTCTG TTATTCTCTG CCTAGTTTTS TSAGAAACAT TTAATAAAG TGGTTTAAAA 3720
 AGAAAAAAA AA 3780

15 12 INFORMATION FOR SEQ ID NO. 1:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 618 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(12) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

Met His Lys Thr Ala Ser Gln Arg Leu Phe Phe Gly Leu Ser Tyr Gln
 1 5 10 15
 25 Asn Ile Lys Ser Ile Met Glu Asp Ser Thr Ile Leu Ser Asp Trp Thr
 20 25 30
 Asn Ser Asn Lys Gln Lys Met Lys Tyr Asp Phe Ser Cys Glu Leu Tyr
 35 40 45
 Arg Met Ser Thr Tyr Ser Thr Phe Phe Ala Gly Val Pro Val Ser Glu
 50 55 60
 30 Arg Ser Leu Ala Asp Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
 65 70 75 80

- 78 -

Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Leu Gly
 85 90 95
 Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser Cys Ser Phe
 100 105 110
 5 Ile Gln Asn Leu Val Ser Ala Ser Leu Gly Ser Thr Ser Lys Asn Thr
 115 120 125
 Ser Pro Met Arg Asn Ser Phe Ala His Ser Leu Ser Pro Thr Leu Glu
 130 135 140
 His Ser Ser Leu Phe Ser Gly Ser Tyr Ser Ser Leu Pro Pro Asn Pro
 10 145 150 155 160
 Leu Asn Ser Arg Ala Val Glu Asp Ile Ser Ser Ser Arg Thr Asn Pro
 165 170 175
 Tyr Ser Tyr Ala Met Ser Thr Glu Thr Ala Arg Phe Leu Thr Tyr His
 180 185 190
 15 Met Trp Trp Leu Thr Phe Leu Ser Trp Ser Glu Leu Ala Arg Ala Gly
 195 200 205
 Phe Tyr Tyr Ile Gly Trp Gly Asp Arg Val Ala Cys Phe Ala Cys Gly
 210 215 220
 Gly Lys Leu Ser Asn Trp Glu Pro Lys Asn Asp Ala Met Ser Glu His
 20 225 230 235 240
 Arg Arg His Phe Pro Asn Cys Phe Ile Leu Glu Asn Ser Leu Glu Thr
 245 250 255
 Leu Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr His Ala Ala Arg
 260 265 270
 25 Met Arg Thr Phe Met Tyr Trp Trp Ser Ser Val Pro Val Gln Pro Glu
 275 280 285
 Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg Asn Asp Asp Val
 290 295 300
 Lys Cys Phe Gly Cys Asp Gly Val Leu Arg Cys Trp Glu Ser Gly Asp
 30 305 310 315 320
 Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu Phe Leu
 325 330 335
 Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile Gln Gly Arg Tyr
 340 345 350
 35 Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Thr Thr Gly Glu
 355 360 365
 Glu Asn Ala Asp Pro Pro Ile Ile His Asn Gly Trp Glu Glu Ser Ser

- 79 -

```

      370              375              380
Ser Glu Asp Ala Val Met Met Asn Thr Pro Val Val Lys Ser Ala Leu
385              390              395              400
Glu Met Gly Phe Asn Arg Asp Leu Val Lys Gln Thr Val Leu Ser Lys
5              405              410              415
Ile Leu Thr Thr Gly Glu Asn Tyr Lys Thr Val Asn Asp Ile Val Ser
      420              425              430
Ala Leu Leu Asn Ala Glu Asp Glu Lys Arg Gln Gln Glu Lys Glu Lys
      435              440              445
10  Gln Ala Gln Gly Met Ala Ser Asp Asp Leu Ser Leu Ile Arg Lys Asn
      450              455              460
Arg Met Ala Leu Phe Gln Gln Leu Thr Cys Val Leu Pro Ile Leu Asp
      465              470              475              480
Asn Leu Leu Lys Ala Asn Val Ile Asn Lys Gln Gln His Asp Ile Ile
15              485              490              495
Lys Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg Val Leu Ile Asp Thr
      500              505              510
Ile Thr Val Lys Gly Asn Ala Ala Ala Asn Ile Phe Lys Asn Cys Leu
      515              520              525
20  Lys Gln Ile Asp Ser Thr Leu Tyr Lys Asn Leu Phe Val Asp Lys Asn
      530              535              540
Met Lys Tyr Ile Pro Thr Gln Asp Val Ser Gly Leu Ser Leu Gln Gln
      545              550              555              560
Gln Leu Arg Arg Leu Gln Gln Gln Arg Thr Cys Lys Val Cys Met Asp
25              565              570              575
Lys Gln Val Ser Val Val Pro Ile Pro Cys Gly His Leu Val Val Cys
      580              585              590
Gln Gln Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Ile
      595              600              605
30  Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
      610              615

```

(2) INFORMATION FOR SEQ ID NO:9:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2691 base pairs

(B) TYPE: nucleic acid

35

- 80 -

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(E) MOLECULE TYPE: cDNA

(F) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

5  ATTTTITAAA TTGATGTCATT AACATTCTAA ACATTTCATCT GTTTTITAAAT AGTAAAAAATT    60
   GAACTTTGGC TTGAATATGT AATGATTGAT TATAACAATT ATGCATAGTC TTTAATAATC    120
   TGCATATTTT ATGCTGCTTT CATGTTTCTC GTAATTAAAG ACTTCACATG TTTAATATTT    180
   ATAATTTTTC TGTGATAGTT TCCATATTTA TATAAAATGA ATACTTAAGA TCAGTAATTC    240
   TCGTCTGTTT GTTATATATG TATTTTCAT CAAAAGACAA AATGGGAGTG AGSTTGAGGC    300
10  TCGTCTGTAAT AGCATTTTCC TAAATATGAA AATGCTTAT CATGATATCT TAGTACTTAT    360
   TTAATATGAA CAAAGACAGC GTGGGCTTTT AATGCTTAT CAAATATCT TCGCATTTAT    420
   GTGAGCTCCA AACTATATAA AAGGAGATA AAAAAAATG TAACTTTTAA AACTTAAGTC    480
   GTTTGTAAT GTAGAGCTCT ACTGTTTACA ATTAAAAATG GTCTTAGTTA TGTGCGCATT    540
   ATTTTITATG CATCACTGCA TAATATATTA GTCTTAGTTA TCAGAAATAG TCTTTATGCT    600
15  TTGTGTTTTS AATTTCTTAA TCCAAATGTC TTTTCTAGA AAGGCTGGAC AAGTCTATT    660
   TTCCAGAGAA GATGACTTTT AACAGTTTTS AAGGACTAG AACTTTTSTA GTTCAGACA    720
   CCAATAGGA TCAAGATTTT GTAGAGAGGT TTAATAGATT AAAAAATTT CCAACTTCC    780
   CAAGTAGTAG TCTGTTTCA GCATCAACAT TCGCCGAGC TGGGTTTCTT TATACGGGTG    840
   AAGGAGACAC CTTGCAATGT TTGAGTCTTC ATGCTGCAAT AGATAGATGG CACTATGGAG    900
20  ACTGAGCTGT TCGAAGACAC AAGGAAATAT CCCCCAATG CAGATTATG AATGTTTCT    960
   ATTTTGAAAA TGGTCTCTCA CAGTCTACA ATCTGTGTAT CCAAAATGCT CAGTCAAAAT    1020
   CTGAAACTG TGTGGAAAT AAAAACTCT TCGCCCTCA TAGGCTACCT GAGACTCATG    1080
   CTGATTATCT GTTGAGAAAT GAGAGTTTGT TAAATATTTT AGGAGGATA TACCCGAGGA    1140
   ACCCTGCAAT GTTATATGAA GAAGCAAAAT TGAAGTAT TGAAGTCTG CCGGACTATG    1200
25  CTCATTTTAC CCCCAGAGAG TTAGCTATG CTGGCTCTTA CTACACAGGG GGTGATGATC    1260
   AAGTGCATG CTTTGTGTGT GGGGAAATG TAAAAAATG GGAACCTCT CATCTGCTCT    1320
   GGTGAGACA CAGGAGACAC TTTCCCAAT GTTTTGTGT TTTGGGCTGG AAGTTTATG    1380
   TTCGAAGTGA ATCTGCTGT AGTTCTGATA GGAATTTCCC AATTTCACA AACTCTCCAA    1440
   GAAATCCAGC CATGGCAGAA TATGAAGCAC GATCCTTAC TTTTGAACA TGCACATCCT    1500
30  CAGTTAACAA GGAGCAGCTT GCAAGAGCTG GATTTTATGC TTTAGGTGAA GCGGATAAAG    1560
   TGAAGTCTCT CCACTGTGGA GAGGGCTCA CCGATTGGA GCAAGTGA GACCCCTGGG    1620
   ACCAGCATGC TAGTCTCTAC CAGGGTCTCA ATACCTATT GATGAGAAG GGGCAAGAAT    1680
   ATATAAATA TATTCATTTA ACCCATCCAC TTGAGGAATC TTTGGGAAGA ACTGCTGAA    1740
   AAACAGGAGC CCAACTTAAA AAAATGGAAT ATACATCTT CCAAAATCT ATGCTGCAAG    1800

```

- 81 -

```

AAGCTATACG AATGGGATTT AGCTTCAAGG ACCTTAAGAA AACAAATGGAA GAAAAAATCC 1850
AAACATCCGG GAGCAGCTAT CTATCACTTG AGGTCCTGAT TGCAGATCTT GTGAGTGCTC 1920
AGAAAGATAA TACGGAGGAT GAGTCAAGTC AAACCTTCATT GCAGAAAGAC ATTAGTACTG 1980
AAGAGCAGCT AAGGCGCCTA CAAGAGGAGA AGCTTTCCAA AATCTGTATG GATAGAAATA 2040
5 TTGCTATCGT TTTTTTCCTT TGIGGACATC TGGCCACTTG TAAACAGTGT GCAGAAGCAG 2100
TTGACAAATG TCCCATGTGC TACACCTCA TTACGTTCAA CCAAAAATT TTIATGTCTT 2160
AGTGGGGCAC CACATGTTAT GTCTCTCTG CTCTAATTGA ATGTGTAAATG GGAGCGAACT 2220
TTAAGTAATC CTGCATTTCG ATTCCAITAG CATCTGTCTG TTTCCAAATG GAGACCAATG 2280
CTAACAGCAC TGTTCCTGTC TAAACATTCA ATTTCIGGAT CTTCGAGTT ATCAGCTGTA 2340
10 TCATTTAGCC AGTGTCTTAC TCGATTGAAA CTTTAGACAG AGAAGCATTT TATAGCTTTT 2400
CACATGTATA TTGTAGTAC ATGCACTTGA TCTGTATATG TAATGCAATT CATCACCTGC 2460
ATGTTTCATG CTTTTTGCAT AAGCTTAAGA AATGAACTGT TGTGTATAAG CATGGAGATG 2520
TGATGGAAATC TGGCAATGA CTCTAATGG CTATTTGTA ACACGGAAAG AACTGCCCCA 2580
CGCTGCTGGG AGGATAAAGA TGTCTTADA TGTCTATTC TGTCTTTAG GATTCTGCCA 2640
15 ATTACTTGG AATTATCGG AATTATAAG TATTATATG ATATTCTGA A 2691

```

1) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 495 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

XL. SEQUENCE DESCRIPTION, SEQ ID NO:11:

```

Met Ile Phe Asn Ser Phe Val Gly Ile Ala Thr Phe Val Leu Ala Asp
25 1 5 10 15
Thr Asn Lys Asp Glu Gln Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
20 25 30
Phe Ala Asn Phe Pro Ser Ser Ser Pro Val Ser Ala Ser Thr Leu Ala
35 40 45
30 Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Gln Cys Phe
50 55 60
Ser Cys His Ala Ala Ile Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val

```

- 82 -

65	70	75	80
Gly Arg His Arg Arg Ile Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe			
	85	90	95
Tyr Phe Glu Asn Gly Ala Ala Gln Ser Thr Asn Pro Gly Ile Gln Asn			
5	100	105	110
Gly Gln Tyr Lys Ser Glu Asn Cys Val Gly Asn Arg Asn Pro Phe Ala			
	115	120	125
Pro Asp Arg Pro Pro Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly			
	130	135	140
10	Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met		
	145	150	155
	Cys Ser Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr		
	165	170	175
Ala His Leu Thr Pro Arg Gln Leu Ala Ser Ala Gly Leu Tyr Tyr Ile			
15	180	185	190
Gly Ala Asp Asp Ala Val Gln Cys Pro Cys Cys Gly Gly Lys Leu Lys			
	195	200	205
Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe			
	210	215	220
20	Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Val Asn Val Arg Ser Glu		
	225	230	235
	Ser Gly Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Ser Pro		
	245	250	255
Arg Asn Pro Ala Met Ala Glu Tyr Glu Ala Arg Ile Val Thr Phe Gly			
25	260	265	270
Thr Trp Ile Tyr Ser Val Asn Lys Ala Gln Leu Ala Arg Ala Gly Phe			
	275	280	285
Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly Gly			
	290	295	300
30	Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Asn Gln His Ala		
	305	310	315
	Lys Cys Tyr Pro Gly Cys Lys Tyr Leu Leu Asp Glu Lys Gly Gln Glu		
	325	330	335
Tyr Ile Asn Asn Ile His Leu Thr His Pro Leu Glu Glu Ser Leu Gly			
35	340	345	350
Arg Thr Ala Glu Lys Thr Pro Pro Leu Thr Lys Lys Ile Asp Asp Thr			
	355	360	365

- 83 -

Ile Phe Gln Asn Pro Met Val Gln Gln Ala Ile Arg Met Gly Phe Ser
 370 375 380
 Phe Lys Asp Leu Lys Lys Thr Met Glu Glu Lys Ile Gln Thr Ser Gly
 385 390 395 400
 5 Ser Ser Tyr Leu Ser Leu Glu Val Leu Ile Ala Asp Leu Val Ser Ala
 405 410 415
 Gln Lys Asp Asn Thr Glu Asp Glu Ser Ser Gln Thr Ser Leu Gln Lys
 420 425 430
 Asp Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu
 435 440 445
 10 Ser Lys Ile Cys Met Asn Arg Asn Ile Ala Ile Val Phe Phe Pro Cys
 450 455 460
 Gly His Leu Ala Thr Cys Lys His Tyr Ala Ile Ala Val Asn Lys Cys
 465 470 475 480
 15 Pro Met Lys Tyr His Val Ile Thr Thr Asn Gln Lys Ile Phe Met Ser
 485 490 495

II. INFORMATION FOR SEQ ID NO:11:

(A) SEQUENCE CHARACTERISTICS:

- (a) LENGTH: 5676 base pairs.
 (b) TYPE: nucleic acid
 (c) STRANDEDNESS: single
 (d) TOPOLOGY: linear

(B) MOLECULE TYPE: cDNA

(C) SEQUENCE DESCRIPTION: SEQ ID NO:11

25 TGGGAGTTCC CCGGAGCCCT GGAGGAAAGC ATGCGAGGTC TGAGCAGCTC TGAGCCCGGC 60
 AGGGTGGGGG CAGTGGCTAA GGCTAGCTG GGGAGGATTT AAAGGATATC CCGCAGCCAG 120
 CCACACCCCA CAGGCGAGGC GAAGGTGCTA CCGCCGAGA TCAGAGGTCA TTCTGGCGT 180
 TCAGAGCCTA GGAAGTGGC TCGGTATTA GCTAGCACT AAAACCGACC AGAAGCCATG 240
 CACAAACTA CATCTCCAGA GAAAGACTTG TCGTTTCTCC TCGCTGTGAT CTCACCATGA 300
 30 ACATGGTTCA AGACAGCGCC TTTCTAGCCA AGCTGATGAA GAGTGTCTGAC ACCTTTGAGT 360
 TGAAGTATGA CTTTCTCTGT GAGCTGTACC GATTCTCCAC GTATTCAGCT TTTCCAGGG 420
 GAGTTCTCTG GTGAGAAAGG AGTCTGGCTC CTGCTGGCTT TTACTACACT GGTGCCAATG 480

- 84 -

	ACAAAGGTCAA	GTGCTTCTGC	TGTGGCCTGA	TGCTAGACAA	CTGGAAACAA	GGGGACAGTC	540
	CCATGGAGAA	GCACAGAAAG	TTGTACCCCA	GCTGCAACTT	TGTACAGACT	TTGAATCCAG	560
	CCACAGCTCT	GGAAAGCTAGT	CCTCGGCCCTT	CTCTTCCTTC	CACGGCGATG	AGCACCATGC	560
	CTTTGAGCTT	TGCAAGTTCT	GAGAATACTG	GCTATTTCAG	TGGCTCTTAC	TCGAGCTTTC	720
5	CCTCAGACCC	TGTGAACCTC	CGAGCAAATC	AAGATTGTCC	TGCTTTGAGC	ACAAGTCCCT	760
	ACCACCTTTC	AATGAACACA	GAGAAGCCCA	GATTACTCAC	CTATGAAACA	TGGCCATTGT	840
	CTTTTCTGTC	ACCAGCAAAG	CTGSCCAAAG	CAGGCTTCTA	CTACATAGGA	CCTGGAGATA	900
	GAGTGGCCTG	CTTTGCGTGC	GATGGGAAAC	TGAGCAACTG	GGAACGTAAG	GATGATGCTA	960
	TGTGAGAGCA	CCAGAGGCCAT	TTCCCCAGCT	GTCCCTTCTT	AAAAGACTTG	GGTCAGTCTG	1020
10	CTTCGAGATA	CAGTGTCTCT	AACCTGAGCA	TGCAGACADA	CGTAGCCCTT	ATTAGAACAT	1080
	TCCTTAAGTC	GCCTTCTAGT	GCAGTAATTC	ATTCCGAGGA	AATTGCAAGT	GGCGGCTTTT	1140
	ATTATACAGG	ACACAGTGTAT	GATGTCAAGT	GTCTTCTCTG	TGATGCTGCG	CTGAGGTGCT	1200
	GGGATCTGTA	AGATGAGCTG	TGGCTGGAAT	ATGCTGAGCT	GTCTTCAAGT	TGTGAGTACT	1260
	TGCTGAGAGT	CAAGAGCTCA	GAATTTCTTA	TGCAAGTTCA	AATTGCTTAC	CCTCATCTAC	1320
15	TTGAGCAGCT	ATTATCTAGC	TGAGACTCTG	CAGAAGATTA	GAATGCAAGC	GCAGCAATCG	1380
	TGCATTTCTG	CTCTGAGAA	AGTTGCGAAG	ATGTCTCTAT	GATGAGCAGC	CCTGTGGTTA	1440
	AAGCAGCCTT	GGAAATGGGC	TTCACTAGGA	GCTTGGTGAG	ACAGAGCCTT	CAGCGGCAGA	1500
	TCTTGGCCAC	TGCTGAGAAC	TACAGGAGCT	TGAGTGACCT	CTTATAGGCC	TTACTCGATG	1560
	CAGAGAGGGA	GATGAGAGAG	GAGCAGATCG	AGCAGGCTGC	CGAGGAGGAG	GAGTCAGATG	1620
20	ATCTAGAGCT	AATGCGGAG	AAAGAAATGG	TGCTTTCTCA	ACATTTGAGC	TGTGTGACAC	1680
	GAATGCTGTA	TTGCTCTCTA	AUTGCAAGGC	CAATCACTTA	ACAGAGCTGC	AATGCTGTGA	1740
	AACAGAAACC	ACACAGCTTA	CAAGCAAGCA	CAGTGATTA	TACTCTCTTA	GCAGAAAGGAA	1800
	AGCTGCAAC	AGCTGATTC	AGAACTCTGC	TTGGGGAAT	TGAGCTTGGC	TTATACAGAG	1860
	ATATATTTGT	GCAGAGGAG	ATTAGGATC	TTCCACAGCA	TGATATTTGA	CTCTACCAA	1920
25	TGGAAGAACA	CTTCCGGAAA	CTCCAGGAGC	AAAGATGTG	TAAAGTGTGT	ATGGACCGAG	1980
	AGGTATCCAT	CTGCTTCTAT	CTCTGTGCTC	ATCTGTCTCT	GTGCAAAAGC	TGCTCTCCCT	2040
	CTCTGAGGAA	GTGTCCCATC	TGTAGAGGGA	GAATCAAGGC	GAGCTGCTGC	ACATTTCTCT	2100
	CCTGAACAAG	ACTAATGGTC	GATGCTCTCA	ACTTCAGGCA	GCAGGAATTT	CAGTGTCACT	2160
	CCCAGCTCCA	TTGCGAAGTT	GAGGCCAGCC	TGATAGCTAC	GAGACAGCCG	CAAGACACACA	2220
30	AATATAAACA	TGAAAAAGTT	TTCTCTGAAG	TCAAGAAATG	ATGAATTACT	TATATAATTA	2280
	TTTTAATTCG	TTTCTTAAAG	AGTGTATTTT	CTTCCCAACT	CAGAAATTTG	TTTTCTGTAA	2340
	ACATATTTAC	ATACTACCTG	CATCTAAAGT	ATTCATATAT	TGATATATTC	AGATCTCATG	2400
	AGAGAGGCTT	TTGTTCTTCT	TCTTGAAAG	CAGGCTTCTC	CTGCACTCTT	GAAATTTCTCA	2460
	GAAAGATTTA	GAATGTTGGC	ATTATGCTTT	CAGAACTTAG	AATCTTCTCT	CGTTGCTTTA	2520
35	AGAAGCCGGA	GCACAGATGT	GAATGCTTTT	TATGTATAGA	AATTTCTCTT	ATTATTTGGA	2580
	TGACATTTTA	OGGATATGAA	ATTTTATATA	AGAATTTCTG	AGAAAAAGTT	AATAAAGCAA	2640
	CACAATTACC	TCTTTTTTTT	TAAAGAAAAA	AAAAAA			2676

- 85 -

(ii) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 600 amino acids

(B) TYPE: amino acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: protein

(iv) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Val Glu Asp Ser Ala Phe Leu Ala Lys Leu Met Lys Asp Ala Asp
10 1 5 10 15
Thr Phe Glu Leu Lys Tyr Asp Phe Ser Cys Glu Leu Tyr Arg Leu Ser
21 25 30
Thr Tyr Ser Ala Phe Phe Arg Gly Val Pro Val Ser Glu Arg Ser Leu
35 40 45
15 Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Ala Asn Asp Lys Val Lys Cys
50 55 60
Phe Cys Cys Gly Leu Met Leu Asp Asn Thr Lys Gln Gly Asp Ser Pro
65 70 75 80
Met Glu Lys His Arg Lys Leu Tyr Pro Ser Lys Asn Phe Val Gln Thr
20 85 90 95
Leu Asn Pro Ala Asn Ser Leu Gln Ala Ser Pro Arg Pro Ser Leu Pro
100 105 110
Ser Thr Ala Met Ser Thr Met Lys Thr Ser Phe Ala Ser Ser Gln Asn
115 120 125
25 Thr Gly Tyr Phe Ser Gly Ser Tyr Ser Ser Phe Pro Ser Asp Pro Val
130 135 140
Asn Phe Arg Ala Asn Gln Asp Cys Pro Ala Leu Ser Thr Ser Pro Tyr
145 150 155 160
His Phe Ala Met Asn Thr Glu Lys Ala Arg Leu Leu Thr Tyr Glu Thr
30 165 170 175
Trp Pro Leu Ser Phe Leu Ser Pro Ala Lys Leu Ala Lys Ala Gly Phe
180 185 190
Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys Asp Gly

```

- 86 -

	195	200	205
	Lys Leu Ser Asn Trp Glu Arg	Lys Asp Asp Ala Met Ser Glu His Gln	
	210	215	220
	Arg His Phe Pro Ser Cys Pro Phe Leu Lys Asp Leu Gly Gln Ser Ala		
5	225	230	235
	Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala Ala Arg		
	245	250	255
	Ile Arg Thr Phe Ser Asn Trp Pro Ser Ser Ala Leu Val His Ser Gln		
	260	265	270
10	Glu Leu Ala Ser Ala Gly Phe Tyr Tyr Thr Gly His Ser Asp Asp Val		
	275	280	285
	Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Tyr Trp Glu Ser Gly Asp		
	290	295	300
	Asp Pro Trp Val Glu His Ala Lys Thr Phe Val Arg Cys Glu Tyr Leu		
15	305	310	315
	Leu Arg Ile Lys Gly Gln Glu Phe Val Ser Gln Val Gln Ala Gly Tyr		
	325	330	335
	Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro Glu Asp		
	340	345	350
20	Glu Asn Ala Asp Ala Ala Ile Val His Phe Gly Pro Gly Glu Ser Ser		
	355	360	365
	Glu Asp Val Val Met Met Ser Thr Pro Val Val Lys Ala Ala Leu Glu		
	370	375	380
	Met Gly Phe Ser Arg Ser Leu Val Arg Gln Thr Val Gln Arg Gln Ile		
25	385	390	395
	Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val Ser Asp Leu Val Ile Gly		
	405	410	415
	Leu Leu Asp Ala Glu Asp Glu Met Arg Glu Glu Gln Met Glu Gln Ala		
	420	425	430
30	Ala Glu Glu Glu Glu Ser Asp Asp Leu Ala Leu Ile Arg Lys Asn Lys		
	435	440	445
	Met Val Leu Phe Gln His Leu Thr Cys Val Thr Pro Met Leu Tyr Cys		
	450	455	460
	Leu Leu Ser Ala Arg Ala Ile Thr Glu Gln Glu Cys Asp Ala Val Lys		
35	465	470	475
	Gln Lys Pro His Thr Leu Gln Ala Ser Thr Leu Ile Asp Thr Val Leu		
	485	490	495

- 87 -

Ala Lys Gly Asn Thr Ala Ala Thr Ser Phe Arg Asn Ser Leu Arg Glu
 500 505 510
 Ile Asp Pro Ala Leu Tyr Arg Asp Ile Phe Val Gln Gln Asp Ile Arg
 515 520 525
 5 Ser Leu Pro Thr Asp Asp Ile Ala Ala Leu Pro Met Glu Glu Gln Leu
 530 535 540
 Arg Lys Leu Gln Glu Glu Arg Met Cys Lys Val Cys Met Asp Arg Glu
 545 550 555 560
 Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val Val Cys Lys Asp
 565 570 575
 10 Cys Ala Pro Ser Leu Arg Lys Tyr Thr Ile Tyr Arg Gly Thr Ile Lys
 580 585 590
 Gly Thr Val Arg Thr Pro Leu Ser
 595 600

15 (ii) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2111 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20

(ii) MOLECULAR TYPE: cDNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGTTATATAA AATAGGAAGT TTTGAAAAAG AAGCTAGCTT CAACCAAAAA GTTTTGGTAA 60
 AACAGTTTCT TAGTCTATTG AGGTACAAA AGAAGGCCAT GTCTTGAAAT GATTCCTTCT 120
 25 TAATTATAAG ACACCTATAG TCGAAAGGSC GTTAAACACA GCGCGACTTT ATAAAAATGCA 180
 GTCTTAGGTT TATGCGCAAA ATACTGTCTC TCGACGAGAT GTATTCACAT GATATATACA 240
 GACTCAAGGT GGTGATATAG AAGATTATAC AGTGAAGGAG TTAACAGTCT GTCTTTAAG 300
 CGCAGTTTCT TTACAGTGAA TACTGTAGTC TTAATAGACC TCAGGTGACT GTGCGAGTTG 360
 ATGTAAGCCA GTTACAGAGAA TACTGTATGA CATCTTCTCT AAGGAAAACC AGCTCCAGAC 420
 30 TTCACTCAGT TCTTTTCATT TCATAGGAAA AGGAGTACTT CAGATGTCAT GTTTAAGTCC 480
 TTATAAGGSA AAGAGCGCTG AATATATGCC CTAGTACCTA GGTTCATATA CTAGTAATAA 540
 GAAGTTAGTT ATGGGTAAAT AGATGTCAGG TTACCCAGAA GATTCATCT CACCCCAAAA 600

- 88 -

	GAGTCTTAAC	TAGTGTCTTG	GCAAGTGAGA	CAGATTTGTC	CTGTGAGGGT	GTCAATTCAC	660
	CAGTCCAGGC	AGAAGACRAT	GAATCTATCC	AGTCAGGTGT	CTGTGCTGGA	GATCTAGTGT	720
	CCAAGTGTGT	AGAAACTTCA	TCTGGAAGTI	TAAGCGGTCA	GAAATACTAT	TACTACTCAT	780
	GGACAAACT	GTCTCCGAGA	GACTCGGCCA	AGGTACCTTA	CACCAAAAC	TTAAACGTAT	840
5	AATGCGAAG	AGCACAATCT	TGTCAAATTC	GACAAAGGAG	AGCGAGGAAA	AAATGAAGTT	900
	TGACTTTTGG	TGTGAATCTC	ACCGAATGTC	TACATATTCA	GCTTTTCCCA	GCGGAGTTCC	960
	TGTCTCAGAG	AGGAGTCTGG	CTCTGCTGG	CTTTTATTAT	ACAGGTGTGA	ATGACAAAGT	1020
	CAAGTGTCTC	TGCTGTGGCC	TGATGTGGA	TAAGTGGAAA	CAAGCGGACA	GTCTGTGTGA	1080
	AAAGCAGAGA	CAGTTCTATC	CGAGCTGCAG	CTTTGTACAG	ACTGTGCTTT	CAGCCAGTCT	1140
10	GCAGTCTGCA	TCTAAGATA	TCTCTGCTGT	GAAAGGTAGA	TTTGCACATT	CGTCACCTCT	1200
	GGAAUGAGGT	GGCATTGACT	CGAATTCTG	CTCTAGGCTT	CTTAATTGTA	GAGCAGTGCA	1260
	AGACTCTGCA	TCTAAGATGG	ATGCTGTAG	CTCTGCTATC	ATTAGAGAG	AGCGCAGATT	1320
	TCTTACTTAT	AGGATGCTG	CTTAATTCT	CTCTGCTATC	ATTAGAGAG	AGCGCAGATT	1380
	CTTCTATTAT	ATAGGCTGCT	GAGAGAGGTT	CTCTGCTATC	ATTAGAGAG	AGCGCAGATT	1440
15	CAACTGGGAA	CGAAAGGATG	ATGCTATGTC	AGAGTACCTG	AGACATTCTC	CCCAGTGTCC	1500
	ATTCTGTGAA	AATACTTCAG	AAAGCAGAGG	CTTTAGTATA	TCAAACTCAA	GTATCCAGAC	1560
	ACACTGTGCT	CGATTGAGGA	CATTCTGTGA	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	1620
	GCAGCTTCCA	AGTCTCTGAT	TCTATTAGCT	CGATCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	1680
	TTGTCTGCTG	GGCTTCTGAT	CTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	1740
20	ATGCTTTTCA	AGTGTCTGAT	TCTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	1800
	TCAAGCTGAA	TATCTCTGAT	CTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	1860
	AGAAATGCTG	GAGCTCTGAT	AGACAGTCTG	CTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	1920
	TCTCTCTGAT	ATGAGCTGAT	CTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	1980
	CTGCTGCTAT	AGTGTCTGAT	CTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	2040
25	CAATGATATT	GTCTCTGAT	TTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	2100
	AAAGCAGAGT	GAAGAGATGG	CATGAGTCTG	CTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	2160
	CTTCTGCTAT	AGTGTCTGAT	CTTCTGCTAT	CTGCTGCTAT	AGTGTCTCTG	TTGAGCGCGA	2220
	AATTACAGAA	CAGGAACATG	ATATTATTAG	AGAGAAAGCA	CAGATACCTT	TACAGCGAAG	2280
	AGAGCTTATT	CAGAGCTGTT	TAGTCAAGGG	AAATGCTCTA	CTTCAATCTT	TCAAAACTTC	2340
30	TCTGAAGGAA	ATTGAGCTCA	CTTATATTGA	AACTTATTAT	GTGAAAGAGA	ATATGAAGTA	2400
	TATTCCAAAC	GAAGAGCTTT	CAGGCTTCTG	ATGCGAAGAG	CACTTCTGGA	GATTACAAGA	2460
	AGAACGAAC	TGCAAGTGT	GTATGGACAG	AGAGCTTCTT	ATTGTGCTCA	TTCTGTGTGG	2520
	TCTCTAGTGA	GTCTGCTAGG	AAATGCTCTT	TTCTGTAGAG	AAATGCTCTT	TCTGCGAGGG	2580
	GACAACTCAG	GGGACTGTGT	GCACATTTCT	CTCTGAGCTG	AGAAATGCTG	TGAAAGTATT	2640
35	CTTGGACATC	AGAAGCTCTG	AGAACAAAGA	ATGAAGTACT	GATTTCAGCT	CTTCAGCAGG	2700
	ACATTCTACT	CTCTTTCAAG	ATTAGTAATC	TTGCTTTATG	AAGGCTAGCA	TTGTATATTT	2760
	AAGCTTAGTC	TGTTGCAAGG	GAAGCTTAT	CTTCTGAGT	TACAGGACTG	TGTCTGTTCC	2820

- 89 -

```

AGAGCAGGAG TTGGGATGCT TGCTGTATGT CCTTCAGGAC TTCTTGGATT TGGAAATTTGT 2880
GAAAGCTTTG GATTGAGGTG ATGTGGAGCT CAGAAATGCT GAAACCAATG GCTCTGGTAC 2940
TCAGTAGTTA GGGTACCCCTG TGGTTCTTGG TGGTTTTCCT TTCTGGAAAA TAAGGATTTT 3000
TCTGCTACTG GTAAATATTC TCTGTTTCTG ACAAATATAT TAAAGTGTTC CTTTAAAGG 3060
5 CCGGCATCAT TGTAGTGTGT GCAGGAGATG ATGCAGGCAA AACACTGTGT ATATAATAAA 3120
TAAATCTTTT TAAAAAGTGT AAAAAAAAAA A 3151

```

(2) INFORMATION FOR SEQ ID NO:14:

(A) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 611 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(E) MOLECULE TYPE: protein

(F) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

15 Met Asp Lys Thr Val Ser Gln Arg Leu Gly Gln Gly Thr Leu His Gln
    1           5           10           15
Lys Leu Lys Arg Ile Met Gln Lys Ser Thr Ile Leu Ser Asn Trp Thr
    21           25           30
Lys Gln Ser Gln Gln Lys Met Lys Phe Asp Phe Ser Cys Gln Leu Tyr
20    35           40           45
Arg Met Ser Thr Tyr Ser Ala Phe Phe Arg Gly Val Phe Val Ser Gln
    51           55           60
Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
    65           70           75           80
25 Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly
    85           90           95
Asp Ser Pro Val Gln Lys His Arg Gln Phe Tyr Pro Ser Cys Ser Phe
   100          105          110
Val Gln Thr Leu Leu Ser Ala Ser Leu Gln Ser Phe Ser Lys Asn Met
30    115          120          125
Ser Pro Val Lys Ser Arg Phe Ala His Ser Ser Pro Leu Gln Arg Gly
   130          135          140

```

- 90 -

Gly Ile His Ser Asn Leu Cys Ser Ser Pro Leu Asn Ser Arg Ala Val
 145 150 155 160
 Glu Asp Phe Ser Ser Arg Met Asp Pro Cys Ser Tyr Ala Met Ser Thr
 165 170 175
 5 Glu Glu Ala Arg Phe Leu Thr Tyr Ser Met Trp Pro Leu Ser Phe Leu
 180 185 190
 Ser Pro Ala Glu Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
 195 200 205
 Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
 210 215 220
 Pro Lys Asp Asp Ala Met Ser Glu His Arg Arg His Phe Pro His Cys
 225 230 235 240
 Pro Phe Leu Glu Asn Thr Ser Glu Thr Glu Arg Phe Ser Ile Ser Asn
 245 250 255
 15 Leu Ser Met Glu Thr His Ser Ala Arg Leu Arg Thr Phe Leu Tyr Trp
 260 265 270
 Pro Pro Ser Val Pro Val Glu Pro Glu Glu Leu Ala Ser Ala Gly Phe
 275 280 285
 Tyr Thr Val Asp Arg Asn Asp Asp Val Lys Cys Phe Cys Cys Asp Gly
 290 295 300
 20 Gly Leu Arg Cys Trp Glu Pro Gly Asp Asp Pro Trp Ile Glu His Ala
 305 310 315 320
 Lys Trp Phe Pro Arg Cys Glu Phe Leu Ile Arg Met Lys Gly Glu Glu
 325 330 335
 25 Phe Val Asp Glu Ile Glu Ala Arg Tyr Pro His Leu Leu Glu Glu Leu
 340 345 350
 Leu Ser Thr Ser Asp Thr Pro Gly Glu Glu Asn Ala Asp Pro Thr Glu
 355 360 365
 Thr Val Val His Phe Gly Pro Gly Glu Ser Ser Lys Asp Val Val Met
 370 375 380
 30 Met Ser Thr Pro Val Val Lys Ala Ala Leu Glu Met Gly Phe Ser Arg
 385 390 395 400
 Ser Leu Val Arg Glu Thr Val Glu Arg Glu Ile Leu Ala Thr Gly Glu
 405 410 415
 35 Asn Tyr Arg Thr Val Asn Asp Ile Val Ser Val Leu Leu Asn Ala Glu
 420 425 430
 Asp Glu Arg Arg Glu Glu Glu Lys Glu Arg Glu Thr Glu Glu Met Ala

- 91 -

```

      435              440              445
Ser Gly Asp Leu Ser Leu Ile Arg Lys Asn Arg Met Ala Leu Phe Gln
      450              455              460
Gln Leu Thr His Val Leu Pro Ile Leu Asp Asn Leu Leu Glu Ala Ser
5  465              470              475              480
Val Ile Thr Lys Gln Glu His Asp Ile Ile Arg Gln Lys Thr Gln Ile
      485              490              495
Pro Leu Gln Ala Arg Glu Leu Ile Asp Thr Val Leu Val Lys Gly Asn
      500              505              510
10 Ala Ala Ala Asn Ile Phe Lys Asn Ser Leu Lys Gln Ile Asp Ser Thr
      515              520              525
Leu Tyr Gln Asn Leu Phe Val Gln Lys Asn Met Lys Tyr Ile Pro Thr
      530              535              540
Glu Asp Val Ser Gly Leu Ser Leu His Ala Gln Leu Arg Arg Leu Gln
15 545              550              555              560
Gln Gln Arg Thr Cys Lys Val Lys Met Asp Arg Gln Val Ser Ile Val
      565              570              575
Phe Ile Pro Cys Gly His Leu Val Val Cys Gln Glu Cys Ala Pro Ser
      580              585              590
20 Leu Arg Lys Cys Phe Ile Cys Arg Arg Thr Ile Lys Gln Thr Val Arg
      595              600              605
Thr Phe Leu Ser
      610

```

(12) INFORMATION FOR SEQ ID NO:15

25 (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 61 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (2) MOLECULE TYPE: Other

(3) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGTGGCGGCTT TTTATTATGT G

21

- 92 -

(3) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGATGACCAAT AAGGAATGAA CACTA

35

10

1. INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Gln Gln Lys Leu Ile Ser Gln Gln Asp Leu

1

5

10

What is claimed is:

Claims

1. A method for enhancing apoptosis in a cell from a mammal with a proliferative disease, said method comprising administering to said cell a compound that inhibits the
5 biological activity of an IAP polypeptide or an NAIP polypeptide, said compound being administered to said cell in an amount sufficient to enhance apoptosis in said cell.
2. The method of claim 1, wherein said cell is proliferating in said proliferative disease.
3. The method of claim 1, wherein said biological activity is the level of expression
10 of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.
4. The method of claim 3, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.
- 15 5. The method of claim 1, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.
6. The method of claim 1, wherein said polypeptide is NAIP.
7. The method of claim 1, wherein said polypeptide is XIAP.
8. The method of claim 1, wherein said polypeptide is HIAP-1.
- 20 9. The method of claim 1, wherein said polypeptide is HIAP-2.
10. The method of claim 1, wherein said compound is a negative regulator of an IAP or an NAIP-dependent anti-apoptotic pathway; wherein said compound is a fragment of said

IAP polypeptide, said fragment comprising a ring zinc finger and having no more than two BIR domains; wherein said compound is a nucleic acid molecule encoding a ring zinc finger domain of said IAP polypeptide; wherein said compound is a compound that prevents cleavage of said IAP polypeptide or said NAIP polypeptide; wherein said compound is a
5 purified antibody or a fragment thereof that specifically binds to said IAP polypeptide or said NAIP polypeptide; wherein said compound is a ribozyme; or wherein said compound is an antisense nucleic acid molecule having a nucleic acid sequence that is complementary to the coding strand of a nucleic acid sequence encoding said IAP polypeptide or said NAIP polypeptide.

10 11. The method of claim 10, wherein said cleavage is decreased by at least 20% in said cell.

12. The method of claim 10, wherein said antibody binds to a BIR domain of said IAP polypeptide or said NAIP polypeptide.

13. The method of claim 10, wherein said nucleic acid sequence encoding said IAP
15 polypeptide or said NAIP polypeptide has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or the nucleic acid sequence of NAIP.

14. The method of claim 10, wherein said antisense nucleic acid molecule decreases the level of said nucleic acid sequence encoding said IAP polypeptide or said NAIP
20 polypeptide by at least 20%, said level being measured in the cytoplasm of said cell.

15. The method of claim 10, wherein said antisense nucleic acid molecule is encoded by a virus vector.

16. The method of claim 10, wherein said antisense nucleic acid molecule is encoded by a transgene.

- 95 -

17. The method of claim 1, wherein said mammal is a human or a mouse.

18. The method of claim 1, wherein said proliferative disease is cancer.

19. The method of claim 18, wherein said cancer is in a tissue selected from the group consisting of ovary, breast, pancreas, lymph node, skin, blood, lung, brain, kidney, liver, nasopharyngeal cavity, thyroid, central nervous system, prostate, colon, rectum, cervix, endometrium, and lung.

20. A method for detecting a proliferative disease or an increased likelihood of said proliferative disease in a mammal, said method comprising:

- (a) contacting an LAP or a NAIP nucleic acid molecule that is greater than about 18 nucleotides in length with a preparation of nucleic acid from a cell of said mammal, said cell proliferating in said disease, said cell from a tissue; and
- (b) measuring the amount of nucleic acid from said cell of said mammal that hybridizes to said molecule, an increase in the amount from said cell of said mammal relative to a control indicating an increased likelihood of said mammal having or developing a proliferative disease.

21. The method of claim 20, wherein said method further comprises the steps of:
(a) contacting said molecule with a preparation of nucleic acid from said control, wherein said control is a cell from said tissue of a second mammal, said second mammal lacking a proliferative disease; and

- (b) measuring the amount of nucleic acid from said control, an increase in the amount of said nucleic acid from said cell of said mammal that hybridizes to said molecule relative to said amount of said nucleic acid from said control indicating an increased likelihood of said mammal having or developing a proliferative disease.

22. The method of claim 20 or 21, said method further comprising the steps of:

- (a) providing a pair of oligonucleotides having sequence identity to or being complementary to a region of said LAP or said NAIP nucleic acid molecule;

- 96 -

- (b) combining said pair of oligonucleotides with said nucleic acid under conditions suitable for polymerase chain reaction-mediated nucleic acid amplification; and
- (c) isolating said amplified nucleic acid or fragment thereof.

23. The method of claim 22, wherein said amplification is carried out using a
5 reverse-transcription polymerase chain reaction.

24. The method of claim 23, wherein said reverse-transcription polymerase chain reaction is RACE.

25. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the
10 nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or the nucleic acid sequence of NAIP.

26. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3.

15 27. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 5.

28. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the
20 nucleotide sequence of SEQ ID NO: 7.

29. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of NAIP.

- 97 -

30. A method for detecting a proliferative disease or an increased likelihood of developing said disease in a mammal, said method comprising measuring the level of biological activity of an IAP polypeptide or a NAIP polypeptide in a sample of said mammal, an increase in said level of said IAP polypeptide or said NAIP polypeptide relative to a sample from a control mammal being an indication that said mammal has said disease or increased likelihood of developing said disease.

31. The method of claim 30, wherein said sample comprises a cell that is proliferating in said disease from said mammal, said cell from a tissue.

32. The method of claim 31, wherein said sample from a control mammal is from said tissue, said sample consisting of healthy cells.

33. The method of claim 32, wherein said mammal and said control mammal are the same.

34. The method of claim 30, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide, or wherein said biological activity is an apoptosis-inhibiting activity.

35. The method of claim 34, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

36. The method of claim 30, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

37. The method of claim 30, wherein said polypeptide is NAIP.

38. The method of claim 30, wherein said polypeptide is XIAP.

- 98 -

39. The method of claim 30, wherein said polypeptide is HIAP-1.

40. The method of claim 30, wherein said polypeptide is HIAP-2.

41. A method for identifying a compound enhances apoptosis in an affected cell that is proliferating in a proliferative disease, said method comprising exposing a cell that
5 overexpresses an IAP polypeptide or a NAIP polypeptide to a candidate compound, a decrease the level of biological activity of said polypeptide indicating the presence of a compound that enhances apoptosis in said affected cell that is proliferating in said proliferative disease.

42. A method for identifying a compound that enhances apoptosis in an affected cell
10 that is proliferating in a proliferative disease, said method comprising the steps of:

(a) providing a cell comprising a nucleic acid molecule encoding a IAP polypeptide or a nucleic acid molecule encoding a NAIP polypeptide, said nucleic acid molecule being expressed in said cell; and

(b) contacting said cell with a candidate compound and monitoring level of biological
15 activity of said IAP polypeptide or said NAIP polypeptide in said cell, a decrease in the level of biological activity of said IAP polypeptide or said NAIP polypeptide in said cell in response to said candidate compound relative to a cell not contacted with said candidate compound indicating the presence of a compound that enhances apoptosis in said affected cell that is proliferating in said proliferative disease.

20 43. The method of claim 42, wherein said cell further expresses a p53 polypeptide associated with said proliferative disease.

44. The method of claim 41 or 42, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an
25 apoptosis-inhibiting activity.

45. The method of claim 44, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.
46. The method of claim 41 or 42, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.
- 5 47. The method of claim 41 or 42, wherein said polypeptide is NAIP.
48. The method of claim 41 or 42, wherein said polypeptide is XIAP.
49. The method of claim 41 or 42, wherein said polypeptide is HIAP-1.
50. The method of claim 41 or 42, wherein said polypeptide is HIAP-2.
51. A method for determining the prognosis of a mammal diagnosed with a
10 proliferative disease, said method comprising the steps of:
(a) isolating a sample from a tissue from said mammal; and
(b) determining whether said sample has an increased level of biological activity of an IAP polypeptide or an NAIP polypeptide relative to a control sample, an increase in said level in said sample being an indication that said mammal has a poor prognosis.
- 15 52. The method of claim 51, wherein said sample comprises a cells that is proliferating in said proliferative disease and said control sample is from said tissue, said control sample consisting of healthy cells.
53. The method of claim 52, wherein said sample and said control sample are from said mammal.
- 20 54. The method of claim 51, wherein said sample further comprises a cell expressing a p53 polypeptide associated with said proliferative disease.

- 100 -

55. The method of claim 51, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.
- 5 56. The method of claim 55, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.
57. The method of claim 51, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.
58. The method of claim 51, wherein said polypeptide is NAIP.
- 10 59. The method of claim 51, wherein said polypeptide is XIAP.
60. The method of claim 51, wherein said polypeptide is HIAP-1.
61. The method of claim 51, wherein said polypeptide is HIAP-2.
62. The method of claim 51, wherein said level is assayed by measuring the amount of IAP peptide of less than 64 kDa present in said sample.
- 15 63. A method for determining the prognosis of a mammal diagnosed with a proliferative disease, said method comprising the steps of:
- (a) isolating a sample from said mammal, said sample having a nuclear fraction; and
 - (b) measuring the amount of a polypeptide that is recognized by an antibody that specifically binds an IAP polypeptide or an antibody that specifically binds an NAIP
- 20 polypeptide in said nuclear fraction of said sample relative an amount from a control sample, an increase in said amount from said sample being an indication that said mammal has a poor prognosis.

- 101 -

64. The method of claim 63, wherein said sample is from a tissue of said mammal, said sample comprising a cell that is proliferating in said proliferative disease, and said control sample is from said tissue, said control sample consisting of healthy cells.

65. The method of claim 64, wherein said sample and said control sample are from
5 said mammal.

66. The method of claim 63, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

10 67. The method of claim 66, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

68. The method of claim 63, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

69. The method of claim 63, wherein said polypeptide is NAIP.

15 70. The method of claim 63, wherein said polypeptide is XIAP.

71. The method of claim 63, wherein said polypeptide is HIAP-1.

72. The method of claim 63, wherein said polypeptide is HIAP-2.

73. The method of claim 63, wherein said amount is measured by immunological methods.

20 74. A method for treating a mammal diagnosed as having a proliferative disease, said method comprising the steps of:

- 102 -

(a) measuring the amount of an IAP or NAIP polypeptide in a first sample from a tissue from said mammal, said first sample comprising a cell that is proliferating in said proliferative disease;

(b) measuring the amount of said polypeptide in a second sample from said tissue.

5 said second sample consisting of healthy cells;

(c) detecting an increase in the amount of said polypeptide in said first sample to the amount of said polypeptide in said second sample; and

(d) treating said mammal with a compound that decreases the biological activity of said polypeptide.

10 75. The method of claim 74, wherein said first sample and said second sample are from said mammal.

76. The method of claim 74, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an
15 apoptosis-inhibiting activity.

77. The method of claim 76, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

78. The method of claim 74, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

20 79. The method of claim 74, wherein said polypeptide is NAIP.

80. The method of claim 74, wherein said polypeptide is XIAP.

81. The method of claim 74, wherein said polypeptide is HIAP-1.

82. The method of claim 74, wherein said polypeptide is HIAP-2.

- 103 -

83. Use of a compound that decreases the biological activity an IAP polypeptide or a NAIP polypeptide for the manufacture of a medicament for the enhancement of apoptosis.

84. The use of claim 83, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

85. The use of claim 84, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

86. The use of claim 83, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

87. The use of claim 83, wherein said polypeptide is NAIP.

88. The use of claim 83, wherein said polypeptide is XIAP.

89. The use of claim 83, wherein said polypeptide is HIAP-1.

90. The use of claim 83, wherein said polypeptide is HIAP-2.

91. A kit for diagnosing a mammal for the presence of a proliferative disease or an increased likelihood of developing a proliferative disease, said kit comprising an oligonucleotide that hybridizes to a nucleic acid sequence that encodes an IAP polypeptide or a NAIP polypeptide.

92. The kit of claim 91, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

93. The kit of claim 91, wherein said polypeptide is NAIP.

1/42

SEQ ID 3—1 GAAAAGGTGGACAACTGCTATTTTCAAGAGAGATGACTTTTAAAGTTTGAAGGATCT 60
SEQ ID 4—1 M T F N S F E G S 9

61 AAACCTTGTGTACCTGACAGCATCATTAAGGAGAGAGATTTTCTAGAGAGATTTAATAGA 120
10 H T C V P A D I N K E E E F V E E F N R 29

121 TTAAAAACTTTTGTAAATTTTCCAACTGGTAGTCTCTGTTTCAGCATCAACACTGGCCACGA 180
30 L K T F A N F P S G S P V S A S T L A R 49

181 GCAGGGTTTCTTTTATCTGGTGAAGGAGATACCCCTGCGGTGCTTTAGTTGTCATGCAGCT 240
50 A G F L V T G E G D T V R C F S C H A A 69

241 GTAGATAGATGGCAATATTCAGACTCAGCAGTTTGGAGAGACACGGAAGATATCCCAAAAT 300
70 V D E W Q Y G D S A V G R H R K V S P N 89

301 TGCAGATTTATCAAGGGTTTATCTTCAAAATAGTGGCAGGCACTCTACAAATTTCTGGT 360
90 C R F I N G F Y L E N S A T Q S T N S 3 109

361 ATCCAGATGCTCAGTACAAATTTCAAAATTTCTTCAAGAGAGAGATTCATTTTGGCTTA 420
110 I Q N G Q Y R V E N Y L G S R D H F A L 129

421 GACAGGCTATCTCAAAATATTAAGATTTTCTTCAAGAGATTCAGGAGTTTATAGATATA 480
130 D R F S E T H A L V L L A T G Q V V D I 149

481 TCAAGACCAATATACCCGAGGAGAGCTTCCATCTATATGAGAGAGAGATTAAGTCC 540
150 S D T I Y P A N P A M Y S E E A R L K S 169

541 TTTCAAGAGCTGGCCAGATATCTCTCACTTACCCCAAGAGAGATTAAGTCTTGGACTC 600
170 H Q N N P D Y A H L T P R E L A S A G L 189

601 TACTAGAGAGATTTTGGTCAAGAGTCACTTCTTTTGTCTGTGGTGGAAACTGAAAAAT 660
190 Y Y T G T G D Q V Q C F C C G G K L K N 209

661 TGGAAAGCTTGTGATCTGTCTCTTCAAGAGAGAGAGCTTCTCAATCTCTCTCT 720
210 M E P Q D E A N S E M R R H F P N C F F 229

721 GTTTTGGGCGGAATTTTAAATTTTCAAGTCAATCTGATCTCTGTGAGTTCTGATAGGAT 780
230 V L G R N L N I R E S D A V S S D R N 249

781 TTCCCAATTCAGCAAAATTTTCAAGAGATTCATCTCTTCAAGATTTATCAAGCAGGATC 840
250 P E K S T N L P S N P S M A D Y E A R I 269

841 TTTAGCTTTTGGAGCTGATATATTAATTAAGAGAGAGCTTTCAGAGAGCTGATTT 900
270 E T F G D W I Y E V N H E Q L A R A G F 289

901 TATGCTTTAGCTCAAGCTGATTAAGTAAAGTGGCTTCACTTGGAGGAGGGCTAAGTAT 960
290 Y A L G E G D K V K C F H C G G G L T D 309

961 TGGAGGCGCACTGAGAGCTCTTGGGAGCAGCATGCTAAATGGTATCCAGGCTGCATATAT 1020
310 W K P S E D P W E Q H A K W Y P G C K Y 329

1021 CTGTTAGAGAGAGAGGAGAGATATATTAAGATATTTCACTTCACTTCACTTCACT 1080
330 L L E Q K G Q E Y I N N I H L C H S L E 349

Fig. 1

SUBSTITUTE SHEET (RULE 26)

11381 GAGTGTCTCTCTTACGACCTAATTACGAAAAACGACGACCTAATTACGAAATTTCTGATCTCT 11400
350 E C L Y A T T E E C P S L T R E I C S C 363

11411 ACCTTTCGAAATTTCTATGTTTACGAAAGCTTATACGAATGCGGCTTCTGATTTCTGAGGACATT 11500
370 C P Q H P M V L E A L R M G F S P K D I 389

11511 AACGAAATTTATCTGAGGAAAAATCTGATATCTCTGATGAAATCTGATTAATTTCTGAGGCTT 11600
390 K K L M E E R L Q L S G S M V K S L E V 409

11611 TTGCTTTCGAGATCTGATGTAATCTCTGAGAAAGACGATATCTGAGATCTGATCTGAGCTGAGCT 11700
410 L V A D L V N A C K D S M L D E S S D T 429

11721 TCTTTACGAAATTTCTGATTTCTGATTTCTGAGAGAGAGCTGAGGCTTCTGAGAGAGAGAGCTT 11800
430 S L Q K E L S T E E Q L R L D E E K L 449

11821 TGCAAAATCTCTATCTGATGAGAAATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCT 11900
450 C K L C N D R N L A I V P V P C G E L V 469

11941 ACTTCTAAAGCAATCTCTGAGAGCTCTGAGAGCTCTCTGAGAGCTCTCTGAGAGCTCTCTGAGAGCT 12000
470 T C K Q C A E A V D R C P Y C Y T V I T 489

12011 TTGAGGCAAAAAATTTCTATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCT 12100
490 P K Q K L F M S * 497

12111 TACTACCTCTGATGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCT 12200
12211 TACGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCT 12300
12311 ACCTTTCGAAATTTCTATGTTTACGAAAGCTTATACGAATGCGGCTTCTGATTTCTGAGGACATT 12400
12411 TTTAAATTTAAAGCAATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCT 12500
12511 ACTTCTAAAGCAATCTCTGAGAGCTCTGAGAGCTCTCTGAGAGCTCTCTGAGAGCTCTCTGAGAGCT 12600
12611 TCTTTTCGAGATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 12700
12711 ACTTCTGAGATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 12800
12811 GAAAGCATAGAGATCTCTTCTGAGAGCTCTCTGATCTCTGATCTCTGATCTCTGATCTCTGATCTCT 12900
12911 TTTAAAGTTTAAAGCAATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13000
13011 AAAAGCTCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13100
13111 AAGATATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13200
13211 AAGTATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13300
13311 TTTAAATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13400
13411 TACGGGCTCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13500
13511 GTATTATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13600
13611 GTTCAAAAGCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13700
13711 GGGGCACTTAACTCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13800
13811 TGTGGATGAAAGATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 13900
13911 TTTCAAAATTAAGTCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 14000
14011 AATTAAATTAAGTCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 14100
14111 ACTTAAAGCTCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 14200
14211 AGAAGCATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 14300
14311 TATGATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCT 14400
14411 ATAGGACAAAACTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCT 14500
14511 GTAGATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCT 14600
14611 AATATCTGGCAAGAAAAAGAGATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCT 14700
14711 AATGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 14800
14811 TACAAAGATCTCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGATCTGAT 14900

Fig 1
Sheet 2 of 3

3/42

```

3161 AATATTGGCAGAAAGAGAAATAGTTGTTTAAATATTTTAAACACACTTGAAATAG 3180
3181 AATCAGTAGGGTAAAGCTAGAGCTTTAAATATGCGCTCATAGAACGTCACAGGGTTTACAT 3180
3182 TACAAGATTCTCACAACAAACCCATTGTAGAGGGTGAGTAAGGCATGTACTACAGAGGAA 3240
3241 AGTTTGAGAGTAAGAACTGTAAATAATATATTTTGTGTACTTTCTAAGAGAAAGAGTA 3300
3301 TTGTTATGTTCTGCTCAACTTCTGTGATTACTACTTTAAGTGATATTTCATTTAAACACT 3360
3361 GCAAAITTTATTTTATTTATTTAAATTTTCTTTTGAGATGGAGTCTTGCTTGTCAACCCAGG 3420
3421 CTGGAGTGCAGTGGAGTGATCTCTGCTCACTGCAACCTCCGCTTCTGGGTTTCAAGCGAT 3480
3481 TCTCGTGCCTCAGCTTCTGAGTAGCTGGAATTACAGGCAGGTGCCACCATGCCCGACTA 3540
3541 AATTTTCTTTATTTTATTTAGTAGAGACGGGGTTTCCACCTGTTTGGCCAGGCTGGTATCAAC 3600
3601 TCTTGACCTCAAGAGATCCACTCGCCTTCCCTCCCAAGTCTGGGATTACAGGCTTGA 3660
3661 GGCACACAGCCCGGCTAAAGCATTTGCAATTTAAATGAGAGCTTTTAAATAATTAATTAAT 3720
3721 AATGCGCTGTTTCTTTTAAATATGTAATCTTCACTTCTTCACTTCTTGCAGTCTGCTGCC 3780
3781 ACTTAGTTTGGTTATATAGTCTATTAAGTCAATTTGGTCTGTATAGTCTAGACTTTAAAT 3840
3841 TTAAGTTTCTTCAAGGGGAGAAAGTGTAAATTTTAAATATCTGTTTCCAGGAGCA 3900
3901 CTTCAGTTCCCAAGTCAGGTAGTCAATCTAGTTTGTAGCCCAAGGACTCAAGGACTG 3960
3961 AATTTCTTCAAGTAAGGCTTTCTCTCTCTGGAGAGCCGCTTCTCAATTAATTTCTTCTA 4020
4021 AATCTTCTATGTTTAAAGTTAAAGCAAGATTTCTTTCTCTCTCTCTCTCTCTCTCTCTCT 4080
4081 TTAATGCAAGAGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4140
4141 TCTTTCAGTTCTTAAATCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4200
4201 AAGGCTTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4260
4261 AATTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4320
4321 AGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4380
4381 TAACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4440
4441 AATTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4500
4501 AATCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4560
4561 TTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4620
4621 AATGGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4680
4681 TTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4740
4741 AATTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4800
4801 TACCTCTTAAACCGGAATCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4860
4861 GGGGCTAGTATATAGTAAATATCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4920
4921 ATGAAATAAATGGGGCTGGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4980
4981 CTGAGGCGGCTGGATCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 5040
5041 CCGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 5100
5101 GCTACTCTGGAGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 5160
5161 GCGAGATCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 5220
5221 AAAAAAAAAA 5111

```

Fig. 1 (cont.)

SUBSTITUTE SHEET (RULE 26)

4/42

111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

Fig. 2

Sequence of the DNA fragment

5/42

3181 ATGAAACTGAGTCCAAAGAGCCAAATGAACAAACACATTAATCTCTGATTATTTATTTA 3240
 3241 AATAGAAATATTTAACTTGTGAAGATCTAATAGTATCATTATACCTAAGCAATCATATTCC 3300
 3301 TGATGATCTATGGGAAATACCTATTATTTAATTAATTTGAAACCAGGTTTTAAGATGTG 3360
 3361 CTAGCCAGTCCCTGTTACTAGTAAATCTCTTTATTTGGAGAGAAATTTAGATTGTTTTGT 3420
 3421 TCTCCTTATTAGAGGATTTGTAGAAAGAAAAAATGACTAATTTGGAGAAAAATTCGGGAT 3480
 3481 ATATCATATTTCACTGAATTCAAAATGTCTTCAGTTGTAAATCTTACCATTTTTCAGT 3540
 3541 ACCTCTAAGAAATAAAAGTGTCTTCTAATTAAAATCATGATGTCATTAAATATGAATACTT 3600
 3601 CTGATTAACAGAGTCTTTAAATAGCCATCTTAGAATCACTGAATATGCGTAATGTACTA 3660
 3661 TTTTCTCTCTTTGAGTNAAGGTCCTTGTGCTTTTNTTCTCTGGGCACTAAATNTCACCATNT 3720
 3721 CCAANRAGCAAAATTAAGCTTATTTGATATTTTGTCTGTGAAACACTTGNACAGCGAGC 3780
 3781 TTTCCCTCCATGNNAGAAGGTTTCATGAGTCACACATTCACATCTTTGGGTTGATGAATGC 3840
 3841 CACTGAACATTTCTAGTAGCCTGGAGNAGTTGACCTACCTGTGGAGATGCCCTGCCATTA 3900
 3901 AATGGCATCTCTGATGGCTTAATACACATCACTCTTCTGTGAGGAGTTTAAATTTTCAACA 3960
 3961 CAGCTTACTCTGTAGCATCATCTTTACATTTGATGTATATAAGATTATACNAAGGTGCAAT 4020
 4021 TGTGTATTTCTTCTTAAATGTATCTGATATAGGATTTAGAACTCTCCATGTTGAAACTCT 4080
 4081 AATGCTATAGAAATAAAATAAATAAAATTTTTCATTTTGGCTTTTTCAGGCTAGTATTA 4140
 4141 AATCTGATAAAGCAAGGCTATGCAAACTACTCTCTAGAGAAAGGCTAGTCTCCCTTT 4200
 4201 TTTTCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4260
 SEQ ID NO: 1 M N I V E N S I F L S N L K 14
 4381 TGAAGAGCGGCAACAGCTTTTGAATGAATAGCACTTGTCTATGTGAATGTACCGAATCT 4440
 15 K S A N T F E L K Y D L S C E L Y R M S 34
 4581 CTAGCTATTCAGCTTTTCTCTGCTGGGCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 4640
 35 T Y S T F P A G V P V S E P S U A R A G 54
 4741 GTTTCTATTACAGTGTGTGAATGACAGGCTCAAAATGCTCTCTCTCTCTCTCTCTCTCTCT 4800
 55 F Y Y T G V N E E V K C F C C G L M L D 74
 4901 ATAACTGGAAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 4960
 75 M N E R G D S F T E K H K K L Y P S C R 94
 5061 GATTCGCTCAGAGCTCTAAATTCCTGTAACAGCTTGGAGGCTAGCTCTCTCAGGCTACTTTT 5120
 95 F V Q S L N S V N N L E A T S Q P T F P 114
 5221 GTTCTTCAGTACAGCTTCCAGAGCACTTCATTAATTCCTGTAACAGCTTGGAGGCTAGCTCT 5280
 115 S S V T H S T H S L L P G T E N S G Y F 134
 5381 TCCCTGCTCTCTTATTCAGCTCTCTGTAACAGCTTGGAGGCTAGCTCTCTCAGGCTACTTTT 5440
 135 R G S Y S N S P S N P V N S R A N Q E F 154
 5541 TTTCTGCTCTGATGAGAGCTTCTCTAGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 5600
 155 S A L M R S S Y P C P M N N E K A R L L 174
 5661 TTTCTTTTCAAGACTGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT 5720
 175 T F Q T W P L T F L S P T D L A R A G F 194
 5821 TTTCTACATAGGAGCTCTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 5880
 195 Y Y I G F G D R V A C F A C G G K L S N 214
 5941 ATTGGCAACGAGGATTAATGATATCTGAGAACTTTGAGAAATTTTCCCAATGCCCCAT 5999
 215 W E P K D N A M S E H L E H F P K C P F 234

Fig. 2 (cont.)

SUBSTITUTE SHEET (RULE 26)

6/42

4921 TTATGGAATTCAGGCTTCAGACACTTCAGATACACAGTTTCTAATCTAGCATGCAGA 4920
335 I E N Q L Q D T S R Y T V S X L S M Q T 354

4981 CACATGCAGCGCGCTTTAAACATTTCTTAACTGGCGCTCTAGGCTTCTAGTTAATCCTG 5040
335 H A A R F K T F F N W P S S V L V N P E 274

5041 AGCAGCTTTCAGACTGCGGCTTTTATTATCTTGGTAAACAGTGAATGCTCAANTGCTTTT 5100
375 Q L A S A G F Y T V G N S D D V K C F C 394

5101 GCTGTCATGGTGGACTCAGGCTTTTGGAACTCTGGAGATGATCCATGGGTTCAACATGCCA 5160
395 C D G G L R C W E S G D D P W V Q H A K 314

5161 ACTGGCTTTCAGGCTTGTGACTCTTCTAAGAAATTAAAGGACAGGAGTTCATCCGCTCAAG 5220
315 W F F R C E Y L I R I K G Q E F I R Q V 334

5221 TTCAAGCCAGTTTACCGCTCTCTATTGAAAGAGTGTGATTCACATCAGACAGCGCCAGGAG 5280
315 2 A S Y F H L L E Q L L S T S D S P G D 354

5281 ATGAAATTCAGAGTCTGCTATCTATCTTTTGAAGTTCAGAGACAGCATTCAGAGAGATG 5340
355 E N A E S S I I R F E P G E D H S E D A 374

5341 CAATCATGATGAATACTCTCTTTTATTAATCTCTGCTGGAAATGGGCTTTAGTAGAAGCC 5400
375 I M M N T F V I H A A V E M G F S R S L 394

5401 TGGTAAAGACAGACAGTTCAGAGAAATCTTACAGACTGAAAGAAATTATAGACTACTCA 5460
395 V E Q T V Q R H I L A T G E N Y R L V N 414

5461 ATGATCTTGTGTAGACTTATTCATATGAAAGATGAAATATAGAGAGAGGAGAGAGAA 5520
415 D L V L D L L M A E C E I R E E E R E R 434

5521 GAGGAGCTTGGGAAAGAGATTAATGATTTATTATTATTCGGAAGATAGATAGGAGC 5580
435 A T E E K E S N D L L L I R K N E M A L 454

5581 TTTTTCAGAGCTTTCAGCTCTGTGATTCAGATCTGATAGCTCTACTAAGTGGCGAATTA 5640
455 F Q H L T C V I P I L D S L L T A G I I 474

5641 TTATGAAACAGAGAGATGATGTTATTAAACAGAGACAGAGAGCTTTTACAGAGCAGAG 5700
475 H E Q E H D V I H Q K T Q T S L Q A R E 494

5701 AAGTGAATGATAGCTTTTATTAAGAAATATTCAGAGAGCTGATTCAGAGAGCTCTC 5760
495 L I D T I L V K G K I A A T V F R N S L 514

5761 TGCAGAGAGCTGAGGCTGTGTTATATGACATTTATTTGTGCAACAGGACATAAATATA 5820
515 2 E A E A V L Y E H L F V Q Q D I X Y I 534

5821 TGGCAGAGAGATGTTTTCAGCTTACCTGAGAGAGAGATTCGAGAGCTTACAGAGAG 5880
535 P T E D V S D L F V E E Q L R R L Q E E 554

5881 AAAGAGATGTTAAGTGTGTATGAGAGAAAGAGTGTGATAGTGTGTTATTCCTGCTGCTC 5940
555 A T C K V C M D K E V S I V F I P C G H 574

5941 ATCTAGTAGTATGCAAGATGCTGCTCTCTTAAAGAAAGTGTGCTTATTCAGAGAGTA 6000
575 L V V C K D C A P S L E H C P I C R S T 594

Fig. 2 (cont.)

SUBSTITUTE SHEET (RULE 26)

7/42

```
6001 CAATCAAGGGGTACAGTTCTGACATTTCTTTTCATGAGAGAAACCAAAACATCGCTAAAC 6060
595 I K G T V R T F L S * 604

6061 TTTCGATTATTTTATTAATGTATATACCTTTTACCTTTTATCCTAATTCGGTTTCCTT 6120
6062 AAAATTTCTTATTTTATTAACACTCAAAACACATTCTTTTGTGTACATATTTATATATGT 6120
6063 ATCTAAACCTATGACATATTTTCTTGAAGATTAAGAGATGATAGGCTTTTGTCTT 6120
6064 ATGACCGAAAGAGGTTGCACTACAAACACATATTCATCAAAATTTCAAGCATTTATG 6120
6065 AAATTTGTAAAGTAAAGTAAAGTAAAGTAAAGTAAAGTAAAGTAAAGTAAAGTAAAGT 6120
6066 TTGGCATTTGTACTAATACCGGGAACATGAAGCCCGGTGTGGTGGTATGTGCTGTAGTCC 6120
6067 CAGGCTGAGGCAAGAGAAATTACTTTAGGCCAGGAGTTTGAATCCATCCTGGGCAAGCATAC 6120
6068 TGAGTCCCTGCTCTTTTAAACAAACAGAGAACAAACAAACAAACAAACAAACAAACAAAC 6120
6069 GTCTTTTTTGTATCAGTGTCTTATACATCGAAGGTGTGCAATATATGTTGAATCAGATTTA 6120
6070 GGGCATGCTGTCTTTTATTAAGATTTCTGTGAGAAATTTTATTAAGCAACCAAAATA 6120
6071 AAAAAAAA 6669
```

Fig. 2 (cont.)

SUBSTITUTE SHEET (RULE 26)

8/42

SEQ ID 7— 1 GAGCGCCCGGCGGATCCGAGCGCGCGGCGCCCTATCTCCCTTGTCCGCGCGCGCTGATTCC 60
 61 CGGCTCTGCGGAGGCGCTTAGGCGAGCGCGCGAGCTTCCGCTGTTTGTGCGCGCGCACTGC 120
 121 GATTTACACCCCTGAGGAATCTCCCTATCCCTATTCTGTCGCCCTGCGATTAATAATCCC 180
 181 ATTATGAGATCTCGAACTTTATAAAGGGATATAGTTTGAATTCATGAGTGTAAATTT 240
 241 TGTGTATGATTAATATTTTAAACATTTGAAGASTTTTCAGAAAGAGGCTAGTAGAGTT 300
 301 GATTAATGATTAATGATTAAGGAGTACTTTTGTGGTAGTACAATATTTTGTAGGCGT 360
 361 TTCTGATACACTAGAAAGGACAAATTTTATCTTGTGATAAATTGATTAATGTTTACAAC 420
 421 ATGACTGATTAATATAGCTGAATAGTCCCTTAATGATGAACAGGCTTATTTAGTTTATAA 480
 481 TGCAGTGTAAAGGTGTGCTGTGGAAATTTTATGGCTAACTAGCTTTATGGAGAAATAC 540
 541 CTTCACTTGATCAAGAAATATAGTGGTATACAAAGTTAGGAAGAAAGTCAACATGATGCT 600
 601 GCAGGAATGGAACAAATACAAATGATATTTAAACAAAGATAGAGTTTACAGTTTGTGAA 660
 661 CTTTAAAGCAATTCATTTGACATCAAGCACTATAGCAGGCGACAGGTTCAACAAAGCTTG 720
 721 TGGTATTTGACCTTCCCGCAAAATTTGTGAGCTGAAGTAAATTTAGCCCACTTAAGTAAATA 780
 781 CTATGATGATTAAGCTCTGTGAATTTAGCTTTTAAATATGTTGACCATATGAAGGTTTAA 840
 841 TTACTTTTGTGATTTGGAATAAATGAGATTTTGTGGTGTGATGTTAAAGTGTCTATA 900
 901 GCGAAAGAGGCTGTGATTAATTTTGTAGCTTTGTGGCAATCAAGTAAATGTTGTCTGTTAT 960
 961 TCAAGCTTTGATAGCTTTTAAACCAATATATAAATAAAGGCAATTTAGGTATTCTATAGT 1020
 1021 TGGTTAGAACTTTGTAAATATAAATCTGTGTGAATAATCAAGGAGTTTAAATATTTTCAG 1080
 1081 AAGTGCATCAAGCTTTGAGGCTTTAAGTTAGTATTAAATCAAGATTATGAACAAATAGC 1140
 1141 ATTATGCTTATTTGAAGATTTATTAACAGCTTCAAGCAATTTGTGCTTCAAGTAGTATCTT 1200
 1201 GGTATCTCAAGATATATTTATTTATCTTGAATTAATTAAGTAAATGCAATTAAGTAAAG 1260
 1261 AGTGTAGTAAATTTGATTAAGATAGATTTGATGATTTGATTTTGTGTGTAATATCTTAG 1320
 1321 TTGATGTGAAGAAATTTTATTTGATGATTTTATGCTATCAACAGTACTGTCACTTACTCA 1380
 M 1
 SEQ ID 8— 1 TGCACAAATGCGCTCCCAAGAGCTTTTCCAGGTCCCTCGTATCAAAACATTAAGAGTA 1440
 1441 H K T A S Q R L F P G P S Y Q N I K S I 21
 1442 TAATGAGATAGCTGAGTCTTGTGATTTGCAACAGGCAACAAACAAATAGAGT 1500
 1443 W S D S T I L E D W T N S N R Q K M R Y 41
 1444 ATGATTTTGTGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGAT 1560
 1445 L F S C E L Y R M S T Y S T F P A G V P 61
 1446 GTGATGAGAAAGAGTCTTGTGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGAT 1620
 1447 C S E R S L A R A G F Y Y T G V N D K V 81
 1448 TCAATGCTTGTGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGAT 1680
 1449 E C F C C G L M L D N W K L G D S P I Q 101
 1450 AAGAGCAATCAAGCTTATATATAGCTTGTGATTTGATGATTTGATGATTTGATGATTTGATGAT 1740
 1451 H H K Q L Y P S C S F I Q N L V S A S L 121
 1452 TGGATGCAATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGAT 1800
 1453 Y S T S K N P E P M R N S F A H S L S P 141
 1454 CCAGCTTGAACATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGAT 1860
 1455 T L E H S S L F S G S Y S S L S P N P L 161
 1456 TTAATCTAGAGCAATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGAT 1920
 1457 M S R A V E D I S S S R T N P Y S Y A M 181
 1458 TGAATCTGAGAAAGCAATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGATTTGATGAT 1980
 1459 S T E E A R F L T Y H M K F L I F L S P 201

Fig. 3

SUBSTITUTE SHEET (RULE 26)

[illegible]

SUBSTITUTE SHEET (RULE 26)

1C/42

```
3061 AATTGAGGAGGTTGCAAGAGAGAGAAATTGTAAGTGTGTATGCAAGAGAGTTTCTG 3120
562 L R R L Q E E R T C K V C M D K E V S V 561

3181 TTGATTTTATTCCTTGTGGTCATCTGCTAGTATGCCAGGATGTGCCCTTTCTCTAAGAA 3180
582 V F I P C G H L V V C Q E C A P S L R K 601

3181 AATGCCCTATTTGCAAGGGGTATAATCAAGGGTACTGTTGCIACATTTCTCTCTTAAAGAA 3240
602 C P I C R G I I K G C V R T F L S * 618

3341 AAATAGTCTATATTTTAACTGTCOMTAAAGAGGTCTTTTAAATATCTGTTGAACACTTGAAG 3360
3361 CCATCTAAGTAAAGAGGGAAATTATGAGTTTTTCACTTAGTAACATTTCATGTTCTAGTCT 3360
3361 GCTTTGCTACTAATATCTTCTTTCTGAAAGATGGTATCTATATTTAATCTTAATCTG 3420
3421 TTTATTTACAGGGGAGACTTATGCTTTGCTGAACATAATAGTATGTATGTGACCTAAG 3460
3461 GGAGTAGCTGTCAGTCTGTTGTTATGCACTCATTTCAAGGAGTTACTGGATTGCTTCTCTTC 3540
3541 AGAAAGCTTTGAATCTAAATTATAGTGTAGAAAGAGCTGGAAACCCGGAGCTCTGGAG 3600
3601 TTCAACAGAGTTATGCTGCTGCTGCTTTGCTTTGCTGCTTTCACTTGTGTTTAAATTAAG 3660
3661 GATTTTCTCTTATTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 3720
3721 AAAAAAAAAA 1732
```

Fig. 3 (cont.)

11/42

SEQ. ID NO. 1 1 ACTCTTTAAATTGATGCAATTAACATTCATAACATTCATCTGTTTAAATAGTAAAAATT 60
 2 GAGCTTTGCGCTTGAATATGTAATGATTCATTTAAGCAATTTATGCAATAGTCTTTAATAATC 120
 3 TGCATATTTTATGCTGCTTCATGTTTCTTAAATTAATGAATTCACATGCTTAAATATT 180
 4 AATACTTTCTGCTGATGTTTTCATTTTATATAAATGAACTACTAGATGCAATATTC 240
 5 TCGCTGCTTTGTTTATATACTATTITTCATCAAAAGACAAATGGGACTGAGGTTGAGGC 300
 6 TCGTTGCTTAAGCACTTTCTTAATGCAAAAGGCGCTATGATGCAATCCCTAGTACTTAT 360
 7 TTAAGTGAGAGAGAAACAGGCTGGGGCTGTAGGTCTGTTAGAGCATGTGTTTGGCATTAT 420
 8 CTGAAGCCCAACACTATAAAGGAGACAAACAAAGCGCAGACTTTAAACATCAAGTG 480
 9 GTTGGGTAATGTACGACTCTACTGTTTAGAATTAAATGTGTCTTAGTTATTGTGCCATT 540
 10 ATTTTATGTCATCACTGCAATACATTAAGTGTCTAGTATCAGAAATAGTCTTATGCT 600
 11 TTGTSTTTGAACTTCTTAATGCAATGTTCTCTTTCTAGAAAAGGTGGACAGTCTTAT 660
 12 TTCCAGAGAGATGCACTTTTACAGCTTTGAGGAGACTAGACTTTTGTCTCTGCAAGCA 720
 SEQ. ID NO. 1 M T F N S F E G T R T F V L A D T 17
 131 CCAATTAAGGATGAAGAATTGTAGAAAGATTTAATAGATTTAAACATTTGCTAACTTCC 780
 14 N K D E E F V E E F H R L K T F A N F P 37
 151 CAAAGTAGTAGTCTGTTTTCAGCAATCAACATTTGGGCGAGGCTGGGTTTCTTTATACCGGTG 840
 16 S S S F V S A S T L A R A G F L Y T G E 57
 171 AAGGACACACCTGCAATGTTTAAATGTCATGTTTAAATATAGATGCAATAGTCTGAG 900
 18 G C T V Q C F R E H A A T D R W Q Y G D 77
 191 ATTCAATTTCTTGAATATCTAAATATTTTAAATTTTAAATTTTAAATTTTAAATTTT 960
 20 C A V G F H F F L S A H C R F I N G F Y 97
 211 ATTTTGAATATGTTGCTGCAAGTCTTACAAATCTGCTATCTGCAAAATGCGGAGTACAAAT 1020
 22 F E N S A A Q S T N P G I Q N G Q Y K S 117
 231 CTGAAGACTGTGTGGGAATGAAATGCTTTTGGCGCTGACAGGCGCACTGAGACTCATG 1080
 24 E N C V G N E N P F A P D R P P E T H A 137
 251 CTGATTTATCTCTTGAAGCTGCAAGCTTTGATGATTTTGAAGACCACTTACTGCAAGCA 1140
 26 C Y L L P T Q Q V V D I S D T I Y P E N 157
 271 ACCCTGCAATGTGTAGTGAAGAACGATGAACTCATTTGCAAGCTGGCGGCACTATG 1200
 28 F A M C E E E A R L K S F Q N W P D Y A 177
 291 CTGATTTAAGCGCTGAGAGATTTAGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1260
 30 H L T P R E L A E A G L Y Y T G A D D Q 197
 311 AAGTGCATGCTTTTCTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTT 1320
 32 T Q C F C C G S R L K N W E P C D R A W 217
 331 GGTGAGACACAGGAGACATTTTCAATGCTTTTCTGTTTGGGCGGCAAGCTTAAATG 1380
 34 S E H R R H F P H C F F V L G R N V N V 237
 351 TTGCAAGTGAATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1440
 36 R S E S G V S S C R N F P N S T N S P R 257
 371 GAATTCAGGCTTGGCAATATGAAAGCAAGGATGCTGCTGCTGCTGCTGCTGCTGCTGCT 1500
 38 N F A M A E Y E A R I V T F G T W T S S 277

Fig. 4

SUBSTITUTE SHEET (RULE 26)

12/42

1 2
 1551 CAGTTAACACAGGAGCGAGCTTGCACAGAGCTGGATTTTATGCTTTAGGTCGAGGCGATAAAG 1560
 279 V M K E Q L A R A G F Y A L G E G D K V 297

1561 TGAAGTGCCTTCCACTGTGGAGGAGGGGCTCAGCGATTGGAAGCCCAAGTGAAGACCCCTGGG 1620
 298 K C F H C G G G L T D W K P S E D P W D 317

2 3
 1621 ACCAGCATGCTAAGTGGTACCCAGGGTGCAAAATACCTATTGGATGAGAAGGGGCAAGAAT 1680
 318 Q H A K C Y P G C K Y L L D E K G Q E Y 337

3 4
 1681 ATATATAATATATTCAITTAACCCATCCACTTGAAGGAATCTTTGGGAAGAAGCTGCTGAAA 1740
 338 I M N I H L T H P L E E S L G R T A E K 357

4 5
 1741 AAACACCCACCGCTAAGTAAAAAATCGATGTACCGATCTTCCAGGATCTCTATGGTGCAG 1800
 358 T P P L T K K I D D T I F Q N P M V Q E 377

1801 AAGCTATACGAATGGGATTTAGCTTCAAGGACCTTAAGAAACATGGAAGAAAAATCC 1860
 378 A I E M G F S F E D L K K T K E E K I Q 397

1861 AAACATCGGGGAGCAGCTATCTATACCTTGAAGGCTCTGATTCAGATCTTGTGAGTGCCT 1920
 398 T S G S S Y L S L E V L T A D L V S A Q 417

5 6
 1921 AGAAGAGATAATAAGAGGATGAGTGAAGTCAAGCTTCAATTCAGAAAGACATTAGTACTG 1980
 418 K D N T E D E P S L T S L Q K D I S T E 437

1981 AAGAGCAGCTAAGGCGGCTACAGAGGAGAGAGCTTCCAAATCTGTATGGATAGAAATA 2040
 438 E Q L R R L Q E E K L S K I C M D R N I 457

2041 TTGCTATGCTTTTCTTTCTTTCTGGACATCTGGGCACTCTGTAACAGTGTGCAGAGCAG 2100
 458 A I V F F P C G H L A T C K Q C A E A V 477

2101 TTGACAAATGTTCATGTGTGTACACCTCTCATTCGCTTCAACCAAAATTTTATGTCTT 2160
 478 D H C P M C Y T V I T F N Q K I F M S 496

2161 AGTGGGGCAGGACATGTATGTTCTTCTTGTCTTAATGCAATGTGTAAATGGGAGCGGACT 2220
 2201 TTAAGTAACCGTGCATTTGGCATTCGATTAAGCATGCTGCTGTTCCAAATGGGAGACCAATG 2280
 2281 CTAACAGCACTGTTTCCGTCTAAACATTCAATTCTGTGATCTTTCGAGTTATCAGCTGTA 2340
 2341 TCATTTCAGCACTGTTTACTGCAATGGAACCTTACACAGAGAGCACTTTATAGCTTTT 2400
 2401 CACATGTATATGGGTATACACTGACTTGCATTTCTATATGTAAAGTGAATTCTACACCTGC 2460
 2461 ATGTTTCATGCTTTTGTATAGCTTAAACAAATGCAAGTCTTCTGTATAACATGGAGATG 2520
 2521 TGATGGAAATCTGCCCCAATGACTTTAAATGGCTTATTTGTAAACACGGGAAGAACTGCCCA 2580
 2581 CGCTGTTGGGAGATAAAGACTGTTTTAGATGCTGCTGCTGCTTTAGGATTTCTGCC 2640
 2641 ATTTACTTGGAAATTTATGGAATTTATATGTACTTATATGATTTTCCGA 2691

Fig. 4 (cont.)

SUBSTITUTE SHEET (RULE 26)

13/42

SEQ. ID 11--1 TGGGAGTTCCCGGAGGCGCTGAGGAAAGGCGCGGAGGCTGTAGCGAGCCCTGAGCCGCGGC 60
61 AGGGTGGGGGCGAGTGGCTAAGGCTTASCTGGGAGGATTTAAAGGTATCGCGCCACCCAG 120
101 CCAACCCCGACAGGCGGAGGCGGAGGCTGCGACCCCGGAGATCAGAGGTCATTGCTGGCGT 180
181 TCAGAGGCTAGGAAGTGGGCTGCGGTATCAGGCTAGCAGTAAACCCGACCAGAAGCCATG 240
241 CACAAAACATACATCCCCAGAGAAAGACTTGTCCCTTCCCGCTCCCTGTCATCTCACCATA 300
301 ACATGGTTCAAGACAGCGGCTTTCTAGCGGAGGCTGATGAAGAGTGGTGCACACCTTTGAGT 360
SEQ. ID 12--1 M V Q D S A F L A K L M K S A D T F E L 20
361 TGAAGTATGACTTTTCCCTGTAGGCTGTAGCGATTTGTCACGCTATTTCAGCTTTTCCCGAGG 420
421 K Y D F S C E L Y R L S T V S A F P R G 40
401 GAGTTCTCTGTGAGAAAGGAGTCTGTCTGCTGCTGCTTTTACTACAGTGGTGGCAATG 480
481 Y P V S E R S L A R A G F Y Y T G A N D 60
481 ACAAGGTCAGTGGCTTCTGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 540
541 K V K C F C C G L M L D N W K Q G D S P 80
541 CCATGGAGAGGACAGAAAGTCTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 600
601 M E K H E R L Y I E C N F Y Q T L N P A 100
601 CCAACAGTGTGGAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 660
661 N S L E A S P R P S L P S T A M S T M P 120
661 CTTTGAGCTTTGGCAAGTGTGTGAGAACTGCGCTATTTTCAGTGGCTCTTACTCGAGCTTTT 720
721 L S F A S S E M T G Y F S G S Y S S F P 140
721 CTTGAGGCTGTGTGAGGCTGCTGAGGAAATCAGATTTGCTGCTGCTGCTGCTGCTGCTGCTGCT 780
781 S D P V H F R A N Q D C P A L S T S P Y 160
781 AGCACTTTGCAATGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 840
841 H F A K N T E R A R L L T Y E T W P L S 180
841 CTTTCTGTGACAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 900
901 F L S P A K L A K A G F Y Y I G P G D R 200
901 GAGTGGGCTGCTTTTGGGCTGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 960
961 Y A C F A C D G K L S N W E R K D D A M 220
961 TGTGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 1020
1021 S E H Q R H F P C C P F L K D L G Q S A 240
1021 GTTGGAGTACAGGCTGTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 1080
1081 S R Y T V S N L S K Q T H A A R I R T F 260
1081 TCTCTAAGTGGGCTTTCTAGTGCAGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1140
1141 S N W P S S A L V H S Q E L A S A G F Y 280
1141 ATTATACAGGAGCAGTGTGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1200
1201 Y T G H S E D V K C F C C D G G L R C W 300
1201 GGGAGTGTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 1260
1261 E S G D L P W V E H A K W F P R C E Y L 320

Fig. 5
SUBSTITUTE SHEET (RULE 26)

14/42

1261 TGGTCAGAAATCAAGAGGCCAAGAAATTTGTCAGGCCAAGTTCAAGCTGGCTATCCTCATCTAC 1320
 321 L R I X G Q E F V S Q V Q A G Y P H L L 340
 1321 TTGAGCAGCTATTATCTACGCTGAGACTCCCCAGAGAGTGGAGATGCAGACGCAGCAATCG 1380
 341 E Q L L S T S D S P E D E N A D A A I V 360
 1381 TGCATTTTGGCCCTGGAGAAAGTTGCGAAGATGTCGTCATGATGAGCAGCGCTGTGGTTA 1440
 361 H F G P G E S S E D V V X M S T P V V K 380
 1441 ARGCAGCCCTTGGAAATGGGCTTCACTAGCAGCCCTGGTGAGACAGACGCTTCAGCGGCAGA 1500
 381 A A L E X G F S R S L V R Q T V Q R Q I 400
 1501 TCGTGGCCACTGGTGAGAACTACAGGACCGTCACTGACCTGCTTATAGGCTTACTCGATG 1560
 401 L A T G E N Y R T V S D L V I G L L D A 420
 1561 CAGAGACCGAGATGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 1620
 421 E D E M R E F L M E Q A A E E E E S D D 440
 1621 ATCTAGCACTAATCCGGAAGAGAGAAATGCTGCTTTTCCAAACATTTGACGTGTGTGACAC 1680
 441 L A L I R E N E M V L F Q H L T C V T P 460
 1681 CATGCTGTATTTGCTTTTAAATTTTAAAGTTCATGATGAGAGAGAGAGAGAGAGAGAGAGAG 1740
 461 M L Y C L L S A R A I T E Q E C N A V K 480
 1741 AACAGAAACACACACATTTATAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 1800
 481 Q X P H T L Q A S T L I D T V L A K G N 500
 1801 ACACTGCAGCAACCTCACTCAGAAACTCCCTTCGGGAATTTGACCCCTGCGTTATACAGAG 1860
 501 T A A T S F R N S L R E I D P A L Y R D 520
 1861 ATATATTTGTCAGACAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 1920
 521 I F V Q Q Q D I R S L P T D D I A A L P M 540
 1921 TGGAGAAAGAGTTTCCGGAAGTCCAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 1980
 541 E E Q L R K L Q E E R K C K V C M D R E 560
 1981 AGGTATCCATCGCTGCTTATCCCTGTGGGATCTGGTCTGTGCAAGAGACTCCGCTCCCT 2040
 561 V S I V F I P C G H L V V C K D C A P S 580
 2041 CTCTGAGGAGAGTGTGCGCATCTGTAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 2100
 581 L R K C P I C R G T I K G T V R T F L S 600
 2101 CCGTGAACAGAGTAAATGTTGATTTTAAAGTTTAAAGTTTAAAGTTTAAAGTTTAAAGTTTAAAG 2160
 2161 CCGAGCTGCTTTGGAACTTGGAGGAGGCTGAGATAGCAGAGACACCGCCAAACACACA 2220
 2221 AATATAACATGAAAACTTTTGTCTGAGAGTCAAGAAATGATGAAATTAATTAATAATAA 2280
 2281 TTTTAATTGGTTTCTTAAAGTGCTATTGCTTCCCAACTCAGAAATTTGTTTCTGTAA 2340
 2341 ACATATTTACATACTAGCTGCATCTTAAGTATTTCATATATTCATATATTCAGATGTCATG 2400
 2401 AGAGAGGGTTTTCTCTTCTGCTGCTGAAAGCAGGGATTGCGCTGCACTCCTGAAATTTCTCA 2460
 2461 GAAAGATTTACAAATGTTGGCATTTATGGTTTCAGAACTAGAATCTTCTCCCGTTGCTTTA 2520
 2521 AGAACCAGGAGCAGAGTGTCCATGCTTTTATGATAGAAATTCCTGTTATTTATTTGG 2580
 2581 TGACATTTTGGGATATGAATTTTATAAGAAATTTTGAGAGAAAGTTTAATTAAGCAA 2640
 2641 CATTAATTAAGCTTTTATTTTAAAGAAAAA 2676

Fig. 5 (cont.)

SUBSTITUTE SHEET (RULE 26)

15/42

SEQ. ID 13—1 AGTTATATATAATACGAAAGTTTTCATAAAGAAAGGCTAGTGCACAGAAAGCCTTTGCTAA 60
 61 AACAGATTCTTAGTTATTTGAGGTAACAAAGAAAGCCCTCTCTGAATTGATTCTGTTCT 120
 121 TATTTATAACAGACTTACAGTGGAAAGGGCCCTTAACACAGGGGGACTTTATAAATGCA 180
 181 GTCTTAGGTTTATGTGCAAAATACTGTCTGTTGACAGATGTATTACACATGATATATA 240
 241 GAGTCAAGGCTGCTGATATAGAAAGATTTACAGTGAAGGAGTTAACAGTCTGTGCTTTAAG 300
 301 CGCAGTTCCCTTTACAGTGAATACTGTAGTCTTAATAGACCTGAGCTGACTGCTGCAGTTC 360
 361 ATGTAACCCCACTTTAGAGAATACTGTATGACATCTTCTCTAAGGAAAGAACAGCTGCAGAC 420
 421 TTCACTCAGTTCCCTTTTCATTTTCATAGGAAAGGAGTAGTTCAGATGTCACTGTTTAAGTCC 480
 481 TTATAAGGGAAGAGCCCTGATATATGCCCCAGTACCTAGGCTTCATTAAGTAACTAATAA 540
 541 GAAGTTAGTTATGGGTAATAAGATCTCAGGTTACCCAGAAAGCTTCATGTGACCCCCAAA 600
 601 GAGTCCCTAATAGTGTCTTGGCAAGTGAAGAGAGATTTGTCCTGTGAGGCTGTCAATTAC 660
 661 CAGTCCAGTAGAAGAGCAATGATCTATCCAGTCAAGTGTGTGTTGGGTGGAGATTTAGTGT 720
 721 CCAAGTGTGAGAACTTCAATCTGGAAGTTTAAAGCGGTCAAGAAATACCTATTACTACTCAT 780
 1 M 1
 781 GGACAAAGCTGTCTCCAGAGACTGGGCCAAGGTACCTTACACCAAAAGCTTAAACGTTAT 840
 SEQ. ID 14—1 D K T V S Q P L S Q S T L E Q K L K R I 21
 941 AATGAGAGAGAGAGAGAGATTTTGTCAAAATTTGACAAAGGAGAGCTGAGAGAAAAATGAAGTT 900
 901 M E H S T I L S N W T K E S E E K M K F 41
 941 TCACTTTTCTTAAGCTGTAAAGATTTTACATATTCAGCTTTTCCCAAGGGGAGTTCC 960
 961 D F S C E L Y F M S T Y S A F P R G V P 61
 941 TGTCTCAGAGAGGAGTGTGTGGCTGTGTGGCTTTTATTATACAGGTGTGAATGACAAAGT 1020
 1021 V S E R S L A R A G F Y Y T G V N D K V 81
 1001 CAAGTGGCTTGTGTGTGGCTGTGTGTGTGATTTGATTAAGTGTGAAAGAGGGGAGCTGCTGTGA 1080
 1081 K C F C C G L M L D N W K Q G D S P V E 101
 1041 AAAGTACAGAGAGATTTTATGAGAGTGTGAGCTTTTACAGAGCTGTGCTTTTACAGCAAGTCT 1140
 1141 H H R Q F Y P S C S F V Q T L L S A S L 121
 1141 CAGTCTCCATTTAGAAATATGTCTTTTGTGAAAGTAGATTTGCAATTCGTCACTCT 1200
 1201 Q S P S K N M S P V K S R F A H S S P L 141
 1001 GGAGAGAGGTGGCAATTAAGTCCAGAGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT 1260
 1261 E R G G I H S N L C S S P L N S R A V E 161
 1061 AAGTTTCTCATCAAGAGATGATTTCTGTGAGCTATGCAATGAGTACAGAGAGAGGAGAGATT 1320
 1321 D F S S R X D P C S Y A M S T E E A R F 181
 1021 TCTTACTTACAGTATGTGTGCTTTAAGTTTCTGTGAGAGAGAGAGCTGGCCAGAGCTGG 1380
 1381 L T Y S M W P L S F L S P A E L A R A G 201
 1001 CTTCCTATTACATAGGGCTGTGAGAGAGAGGTGGGCTGTGTTTGGCTGTGTGTGAGAACTGAG 1440
 1441 F Y Y I G P G D R V A C F A C G G K L S 221
 1441 CAAGTGGAAAGCAAGAGATGATTTATGTAGAGAGAGAGAGAGAGATTTTCCCACTGTCT 1500
 1501 N W E P H D D A M S E H R R H F P H C P 241
 1001 ATTCTGGAAGATATCTTCAAGAAACAGAGAGGTTTAGTATATCAATCTAAGTATGCAGAC 1560
 1561 F L E N T S E T Q R F S I S N L S M Q T 261

Fig. 6

SUBSTITUTE SHEET (RULE 26)

15/42

1561 ACACCTCTGCTCGATTGAGGACATTTCTGTACTGGCCACCTAGTGTTCCTGTTCCAGCCCCGA 1620
 262 H S A R L R T F L Y W P P S V P V Q P E 281
 1621 GCAGCTTGCAAGTGCCTGGATTCTATTACGTGGATCGCRATGATGATGTCAAGTGCCTTTTG 1680
 282 Q L A S A G F Y Y V D R N D D V K C F C 301
 1681 TTCTGATGGTGGCTTGGAGTCTTGGGAACTGGAGATGACCCCTGGATAGAACACGCCCA 1740
 302 C D G G L R C W E P G D D P W I E H A K 321
 1741 ATGCTTTCCAGGTTGTGAGTCTCTGATACGGATGAGGGTCCAGGAGTTTGTTCATGAGAT 1800
 322 W F P R C E F L I R M K K G Q E F V D E I 341
 1801 TCAAGCTAGATACTCTCATCTCTCTGAGCAGCTGTGTGCCACTTCAGACACCCAGGAGA 1860
 342 Q A R Y P H L L E Q L L S T S D T P G E 361
 1861 AGAAATGCTGACCTTACAGAGACASTGGTGCATTTTGGCCCTGGAGAACTTCGALAGA 1920
 362 E N A D F T E T V V H F G P G E S S K D 381
 1921 TCTGCTCATGATGAGCAGCAGCTTCTGTTTAAAGCAGCTTTGAAATGGGCTTCAGTAGGAG 1980
 382 V V M M E T F V Y H A A L E M G F S R S 401
 1981 CTTGCTGAGCAGAGAGCTTCTGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 2040
 402 L V R Q T V Q R Q I L A T G E N Y R T V 421
 2041 CATGATATTGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2100
 422 N D I V S V L L N A E D E R R E E E K E 441
 2101 AAGACAGAGCTGAGAGAGATGGATGAGAGTGCATGATGATGATGATGATGATGATGATGATGAT 2160
 442 R Q T E E M A S G D L S L I R R N R M A 461
 2161 CTTCTTTTCAAGCTTGCAGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2220
 462 L F Q Q L T H V L P I L D N L L E A S V 481
 2221 AATTGCAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 2280
 482 T I K Q E H D I I R Q K T Q I P L Q A R 501
 2281 AGAGCTTATTGACAGCTGCTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2340
 502 E L I D T V L V H G N A A A N I F K N S 521
 2341 TCTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 2400
 522 L K E I D S T L Y E N L F V E K N M K Y 541
 2401 TATTGCAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 2460
 542 I P T E D V S G L S L E E Q L R R L Q E 561
 2461 AGAGCGAAGCTTGCAGAGTGTCTATGAGACAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 2520
 562 E R T C K V C M D R E V S I V F I P C G 581
 2521 TCATCTAGTAGTCTGCGAGGATGTGCGCGCTTCTCTAAGGAGTGTGCGCGCTTCTCTAAGGAGTGTG 2580
 582 H L V V C Q E C A P S L R K C P I C R G 601
 2581 GACAATCAAGGGAGTGTGCGAGATTTCTCTCATGAGTGAAGAGATGTCTCTGAAGTATT 2640
 602 T I K G T V R T F L S 612

Fig. 6 (cont.)

SUBSTITUTE SHEET (RULE 26)

17/42

```
2641 GTTGGACATCAGAAAGCTGTCAGAACAAAGAAATGAACTACTGATTTTCAGCTCTTCAGCAGG 2700
2701 ACATTCTACTCTCTTTCAGATTAGTAATCTTGCTTTATGAGGGTAGCATTGTATATTT 2760
2761 AAGCTTAGTCTGTGTCAGGGAGGGTCTATGCTGTTCAGCTACAGGACTGTGTCTGTTC 2820
2821 AGAGCAGGAGTTGGGATGCTTGCTGTATGTCTTCAGGACTTCCTGGATTTCGAATCTGT 2880
2881 GAAAGCTTTGGAATTCAGGTGATGTGAGCTCAGAAATCCTGAAACCAAGTGGCTCTGCTAC 2940
2941 TCAGTAGTTAGGGTACCCCTGTCTCTTGGTGCTTTTCCCTTCGGAATAAGGATTTT 3000
3001 TCTGCTACTGGTAAATATTTTCTGTCTGTCAGAAATATATTAAAGTGTCTCTTTAAAGG 3060
3061 CGTGCATCATTTGATGTGTGCAGGGATGTATGCAGGCAAAACACTGTGTATATATAATA 3120
3121 TAAATCTTTTAAAAAGTGTAAAAA 3151
```

Fig. 6 (cont.)

SUBSTITUTE SHEET (RULE 26)

18/42

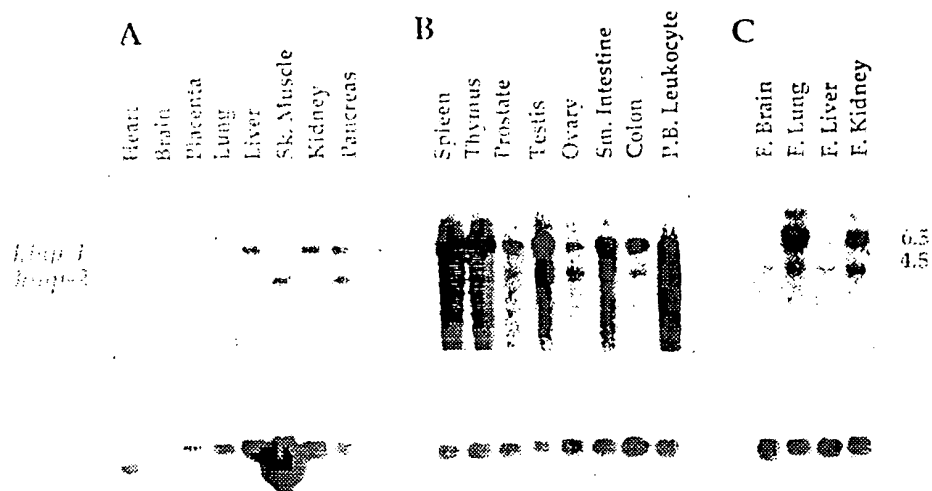


Fig. 7

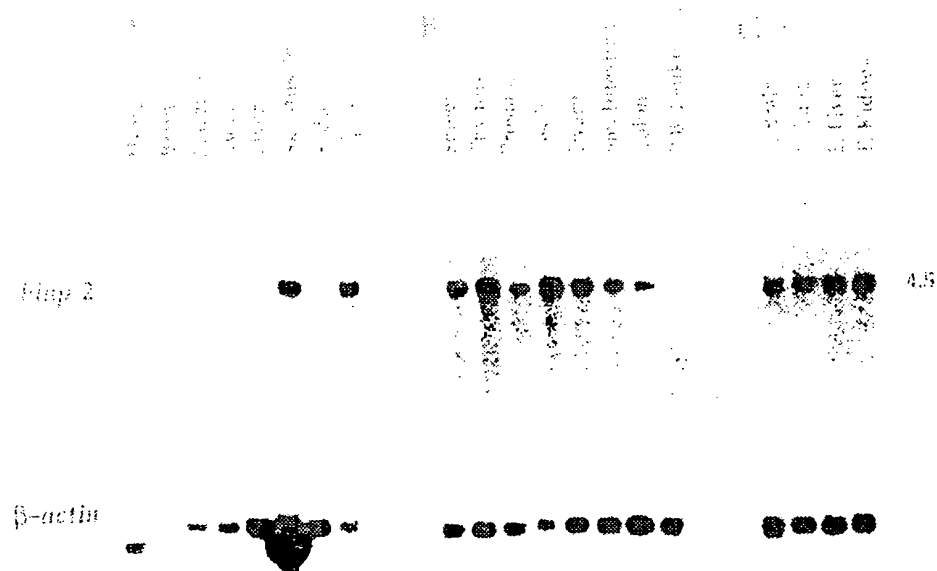


Fig. 8

SUBSTITUTE SHEET (RULE 26)

19/42

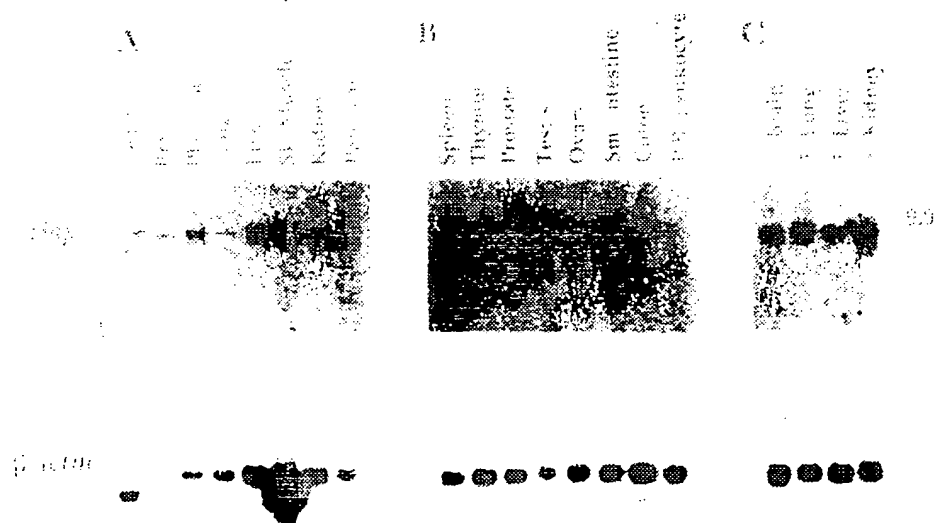


Fig. 9

SUBSTITUTE SHEET (RULE 26)

20/42

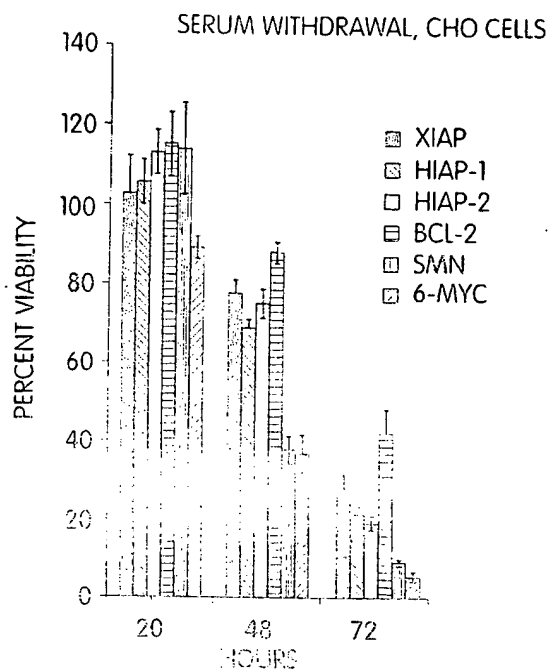


Fig. 10A

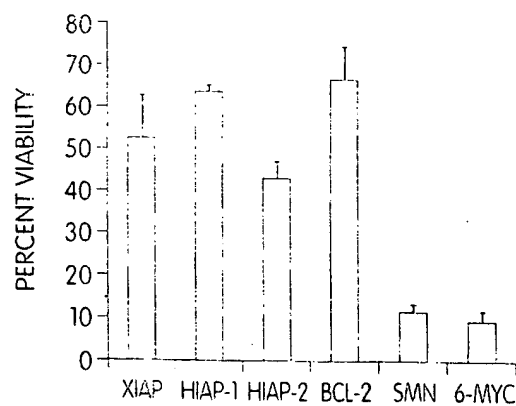
MENADIONE (20 μ M), CHO CELLS. 24hr SURVIVAL

Fig. 10B

SUBSTITUTE SHEET (RULE 26)

21/42

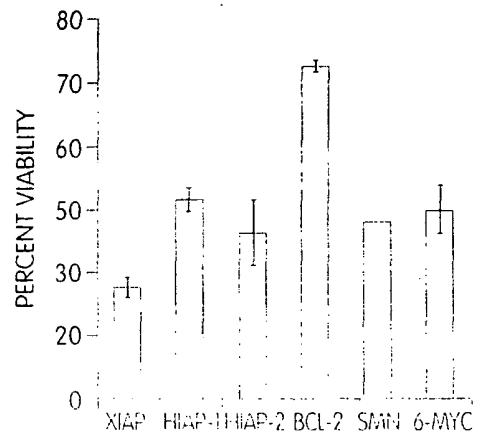
STAUROSPOURINE (1 μ M), RAT-1 CELLS, 24 HOUR SURVIVAL

Fig. 10C

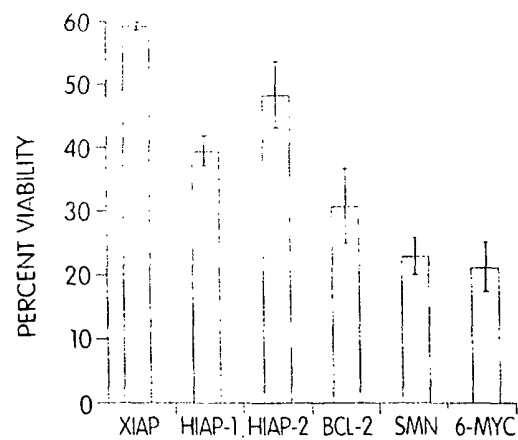
MENADIONE (10 μ M), RAT-1 CELLS, 18 HOUR SURVIVAL

Fig. 10D

SUBSTITUTE SHEET (RULE 26)

22/42

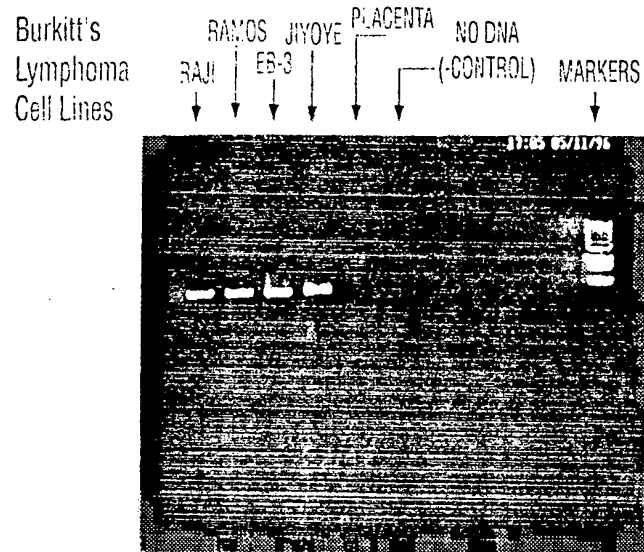


Fig. 11

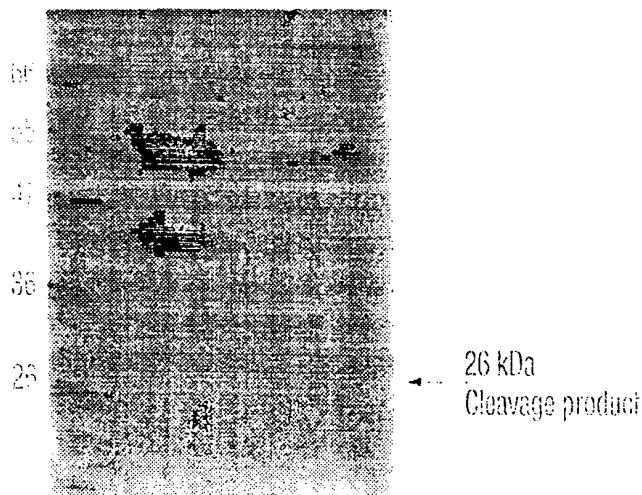


Fig. 12

SUBSTITUTE SHEET (RULE 26)

23/42

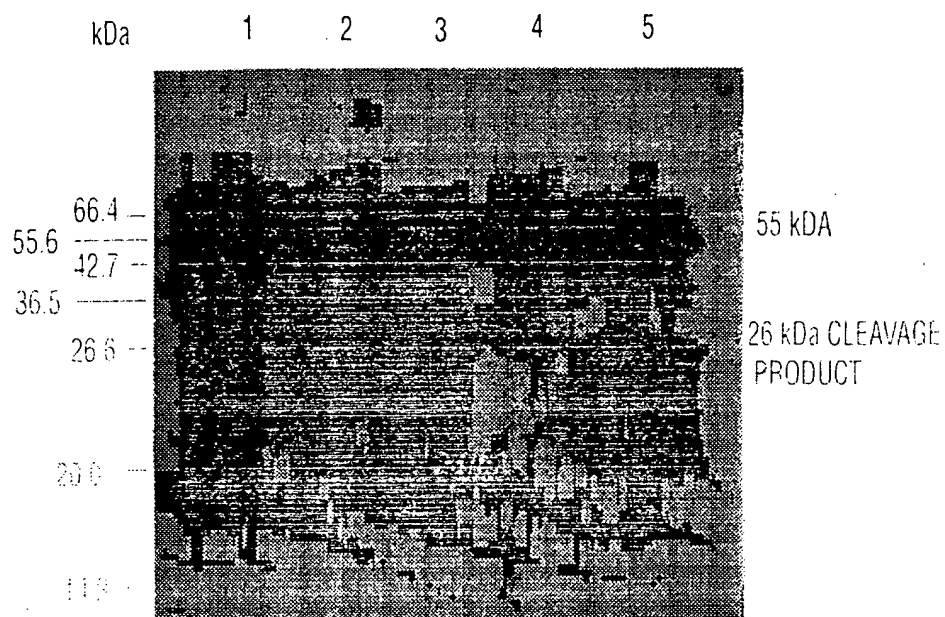


Fig. 13

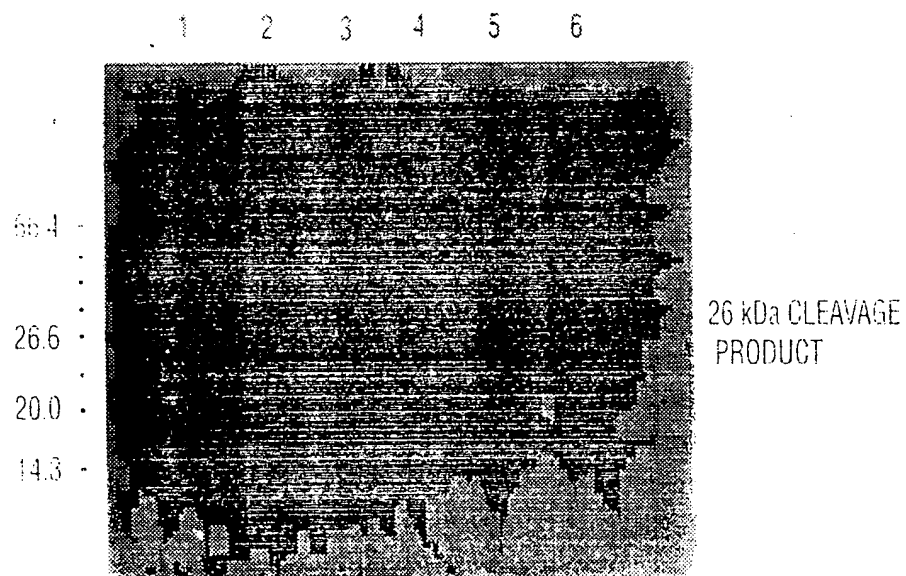


Fig. 14

SUBSTITUTE SHEET (RULE 26)

24/42

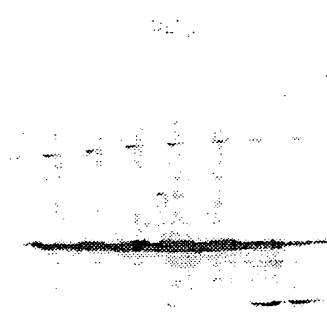


Fig. 15A

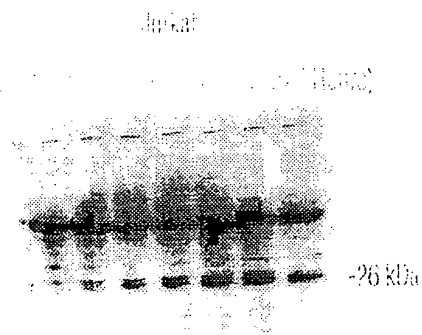


Fig. 15B

SUBSTITUTE SHEET (RULE 26)

25/42

3 HOURS
HOURS 0 3 7 Cyto. Nucl.

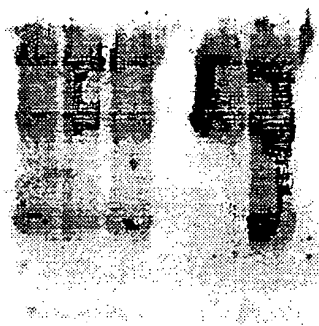


Fig. 16A

3 HOURS
MARKER 0 3 7 Cyto. Nucl.

97.2
66.4
55.6
42.7
36.5
26.6



← 55 kDa
← 25 kDa

Fig. 16B

SUBSTITUTE SHEET (RULE 26)

26/42

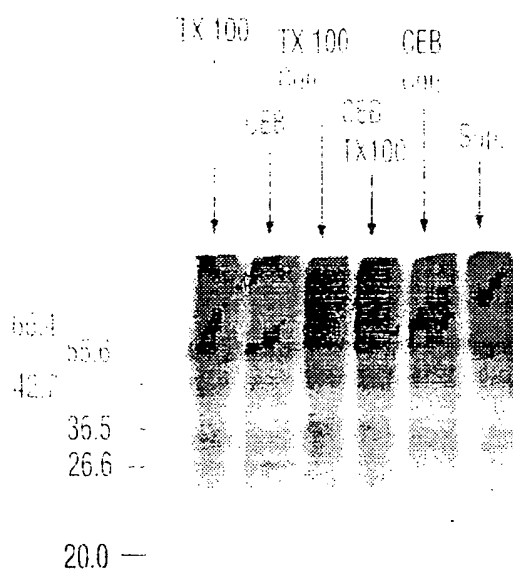


Fig. 17

SUBSTITUTE SHEET (RULE 26)

27/42

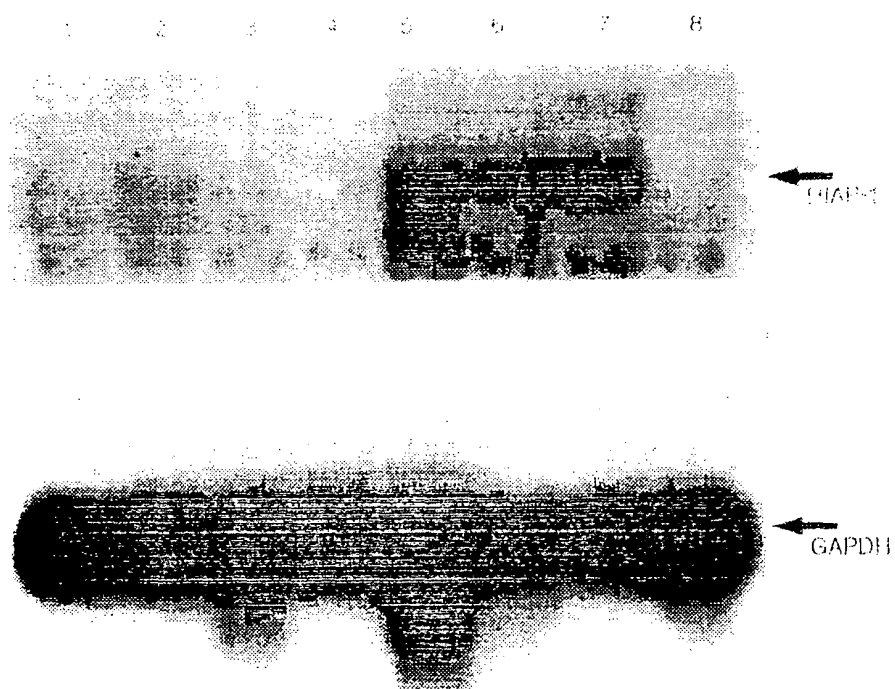


Fig. 18

SUBSTITUTE SHEET (RULE 26)

28/42

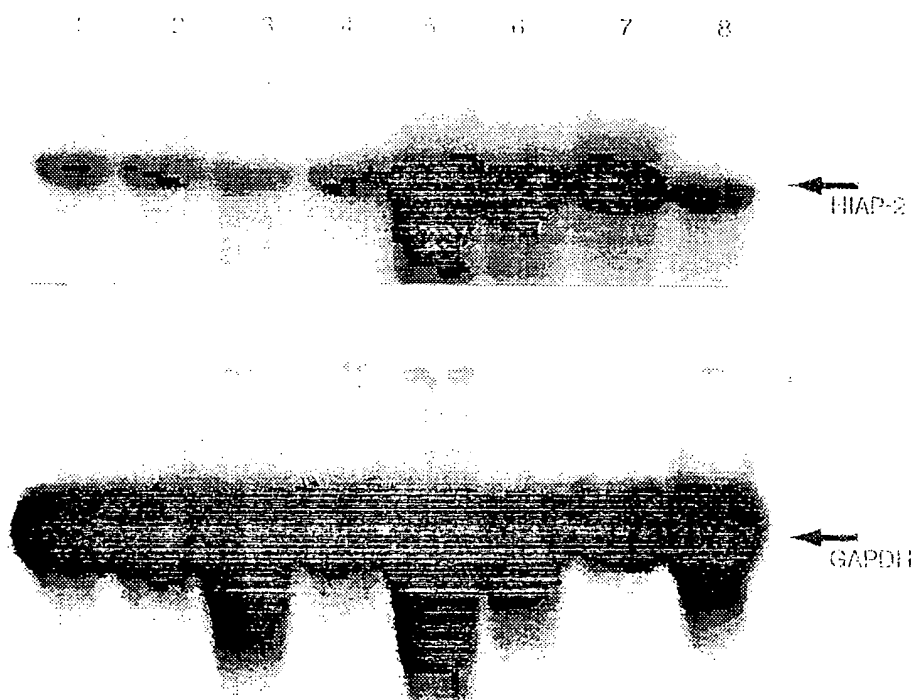


Fig. 19

SUBSTITUTE SHEET (RULE 26)

29/42

TOXICITY OF TAXOL ON DNA FRAGMENTATION IN
CISPLATIN-SENSITIVE (OV2008) AND -RESISTANT (C13)
HUMAN OVARIAN EPITHELIAL CANCER

C13 OV2008



0 1 0 1

TAXOL CONCENTRATION (μ M)

Fig. 20

SUBSTITUTE SHEET (RULE 26)

30/42

SELECTIVE INFLUENCE OF CISPLATIN ON DNA FRAGMENTATION
IN SENSITIVE (OV2008) AND RESISTANT (C13)
HUMAN OVARIAN EPITHELIAL CANCER

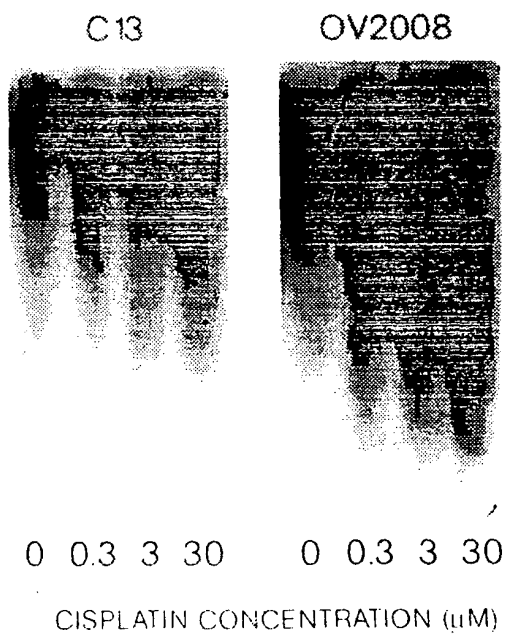


Fig. 21

SUBSTITUTE SHEET (RULE 26)

31/42

EFFECTS OF TAXOL ON XIAP AND HIAP-2 PROTEIN CONTENT IN
CISPLATIN-RESISTANT (C13) AND -SENSITIVE (OV2008)
HUMAN OVARIAN EPITHELIAL CANCER CELLS IN VITRO

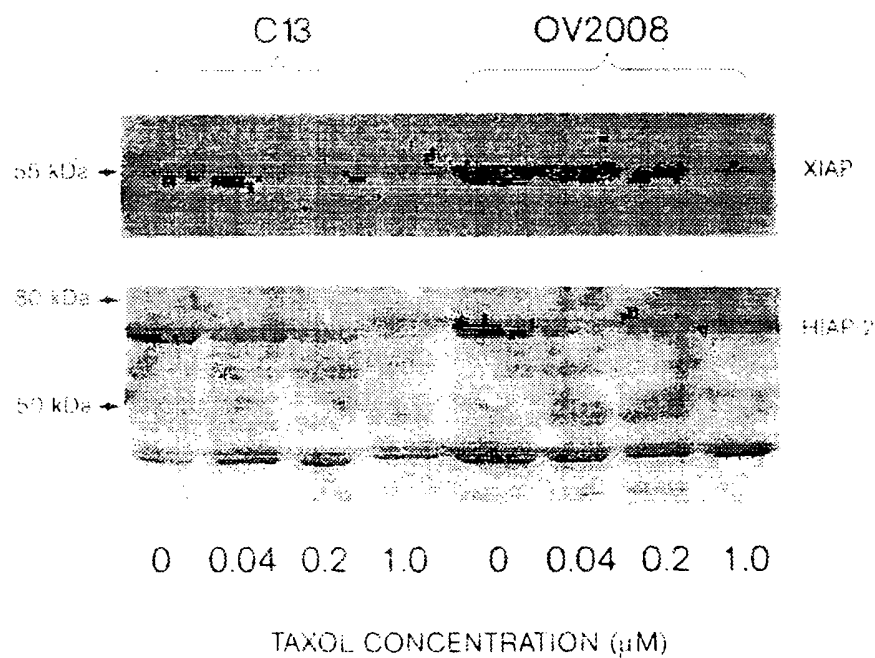


Fig. 22

SUBSTITUTE SHEET (RULE 26)

32/42

INFLUENCE OF TAXOL and TGF β ON HIAP-2 mRNA ABUNDANCE
IN CISPLATIN-SENSITIVE (OV2008) AND -RESISTANT (C13)
HUMAN EPITHELIAL CANCER CELLS IN VITRO

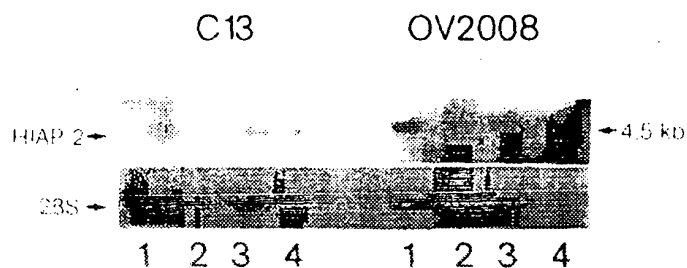


Fig. 23A

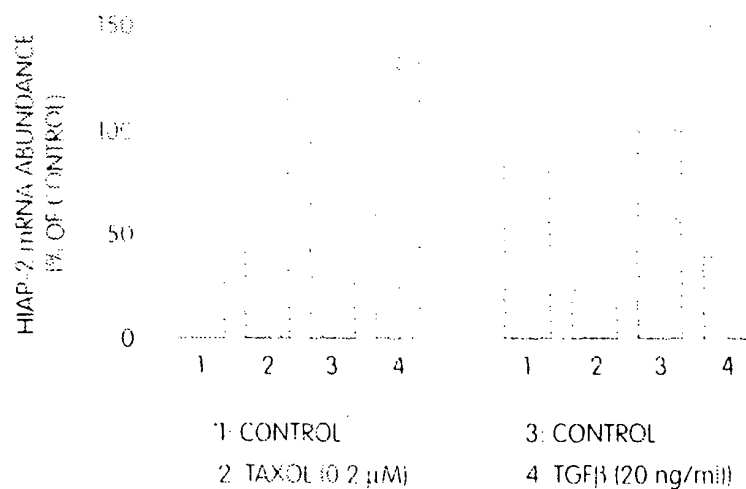


Fig. 23B

SUBSTITUTE SHEET (RULE 26)

33/42

INFLUENCE OF TGF β ON XIAP PROTEIN EXPRESSION AND DNA
FRAGMENTATION IN CISPLATIN-SENSITIVE (OV2008) AND -RESISTANT (C13)
HUMAN OVARIAN EPITHELIAL CANCER CELLS IN VITRO

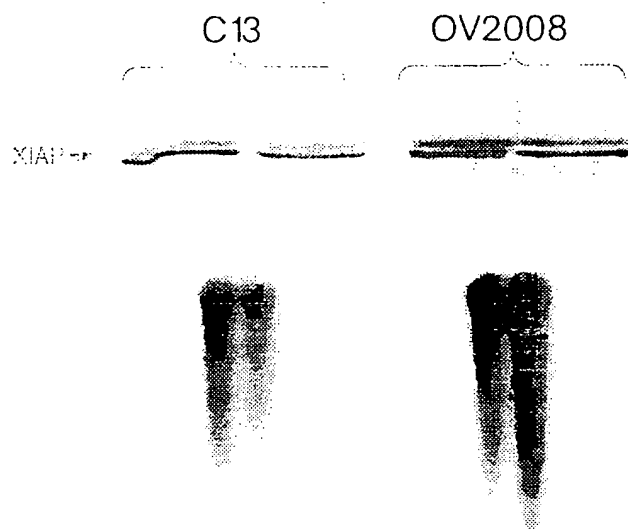


Fig. 24A

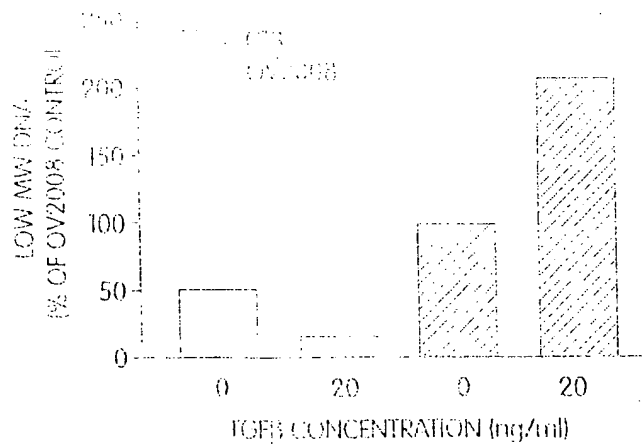


Fig. 24B

SUBSTITUTE SHEET (RULE 26)

34/42

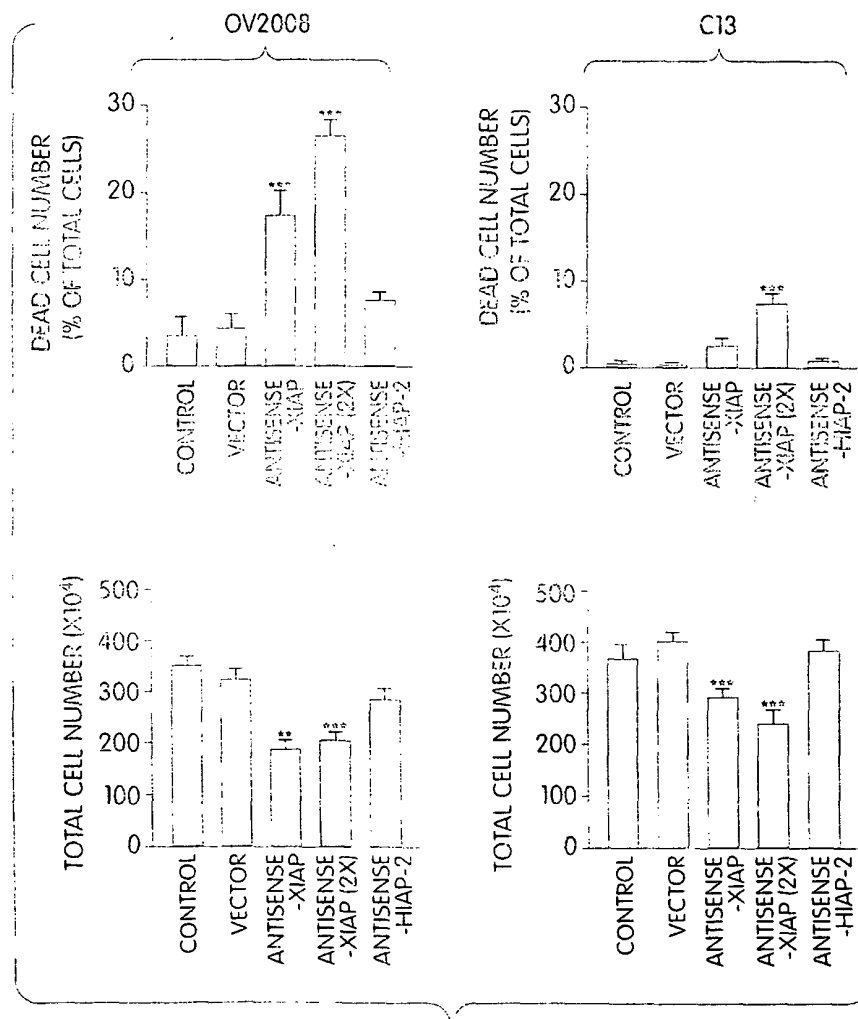


Fig. 25

SUBSTITUTE SHEET (RULE 26)

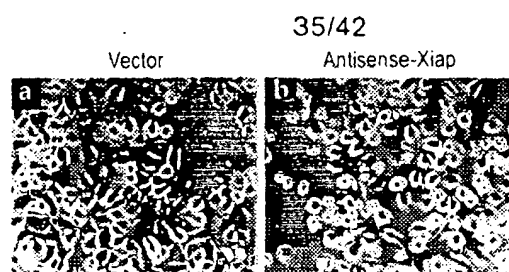


Fig. 26A

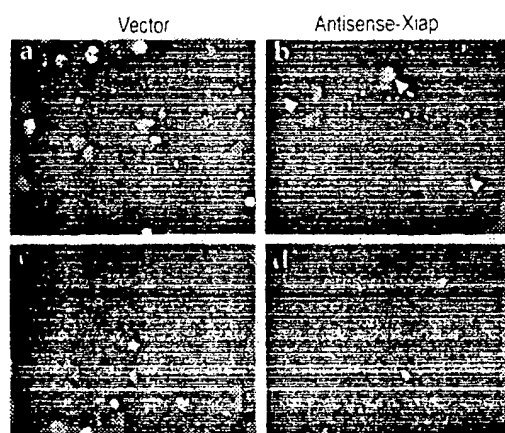


Fig. 26B

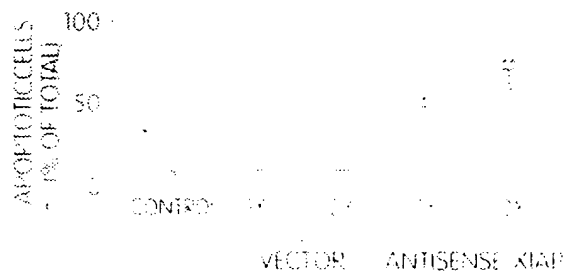


Fig. 26C

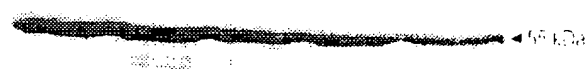


Fig. 26D

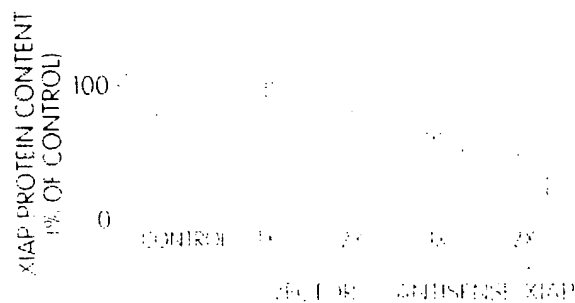


Fig. 26E

SUBSTITUTE SHEET (RULE 26)

36/42

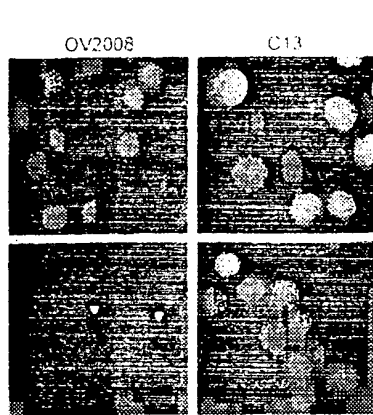


Fig. 27A



Fig. 27B

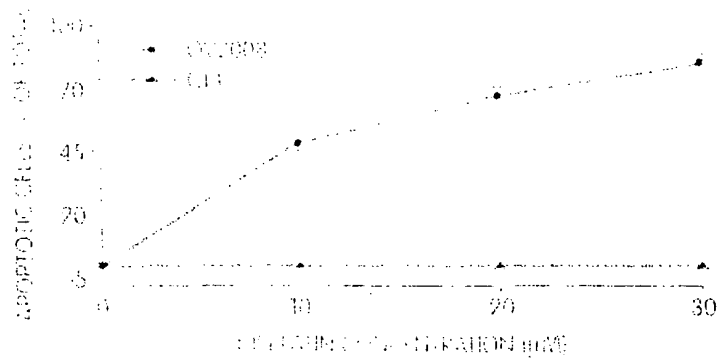


Fig. 27C

SUBSTITUTE SHEET (RULE 26)

37/42

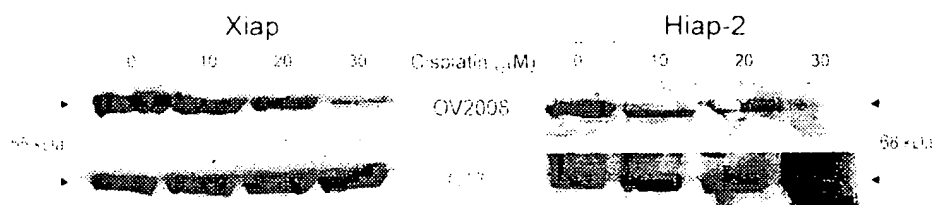


Fig. 28A

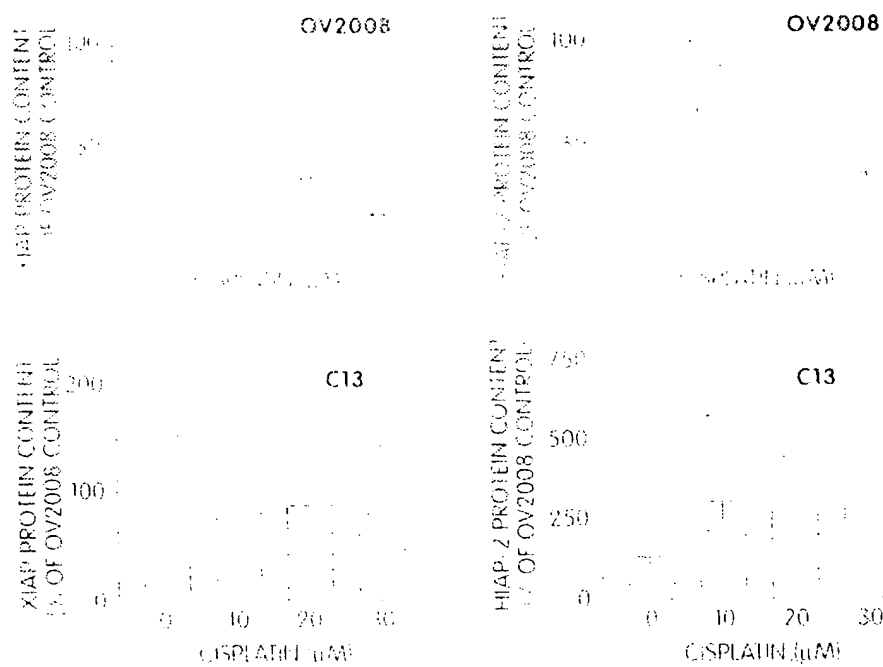


Fig. 28B

SUBSTITUTE SHEET (RULE 26)

38/42

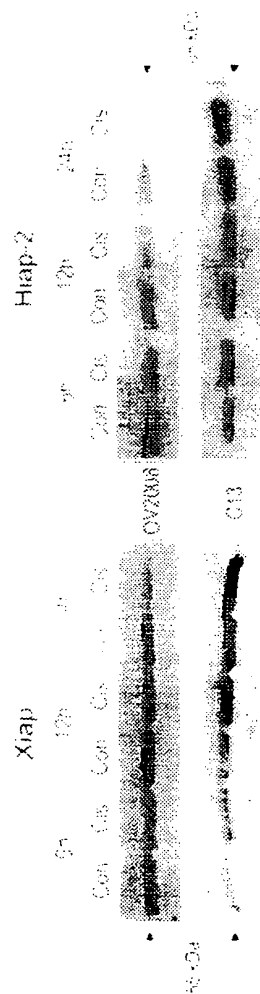


Fig. 29A

SUBSTITUTE SHEET (RULE 26)

39/42

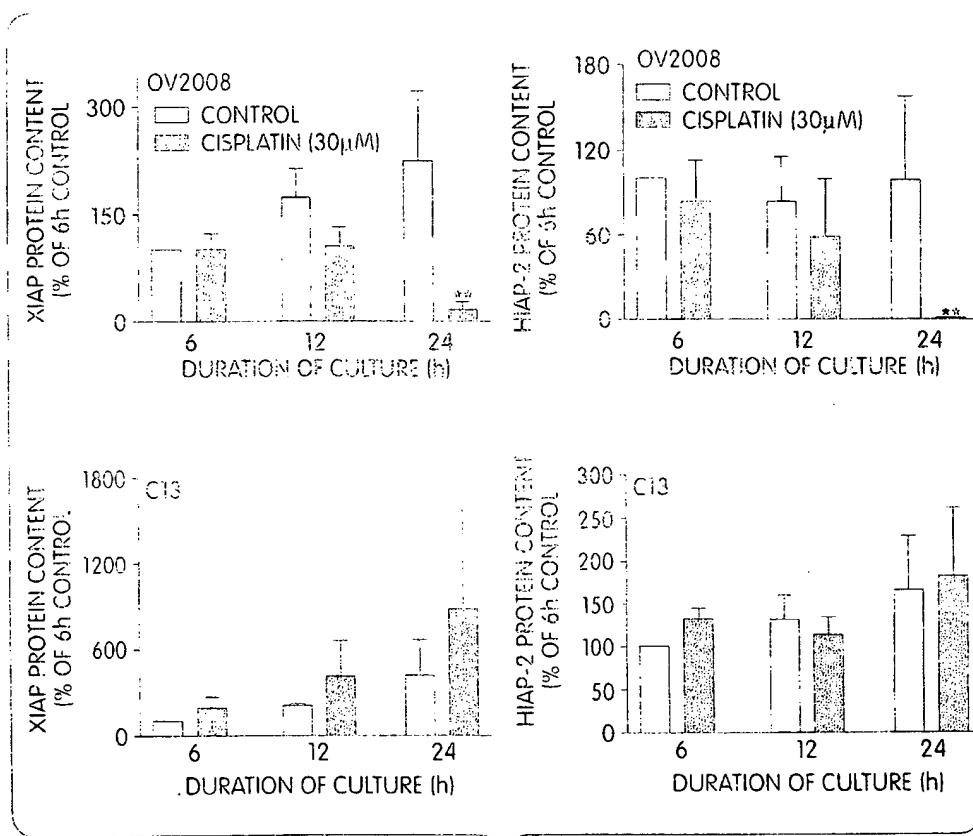


Fig. 29B

SUBSTITUTE SHEET (RULE 26)

40/42

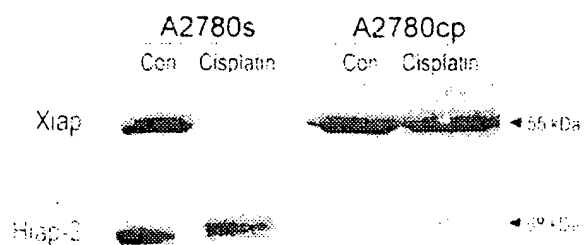


Fig. 30A

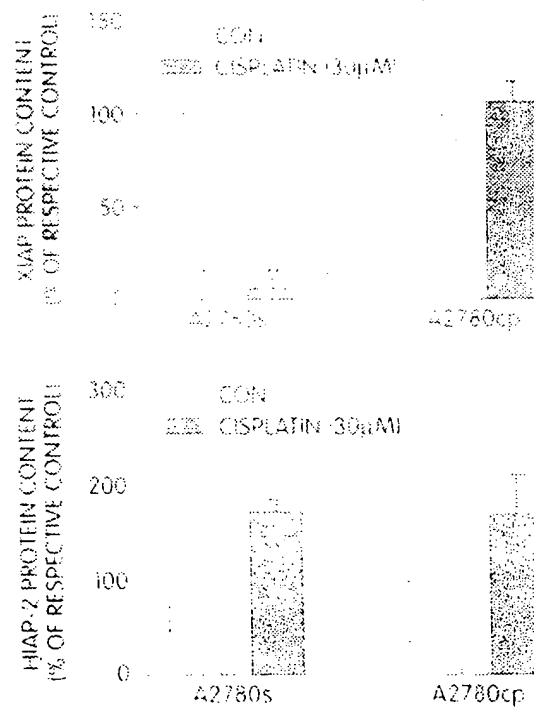


Fig. 30B

SUBSTITUTE SHEET (RULE 26)

41/42

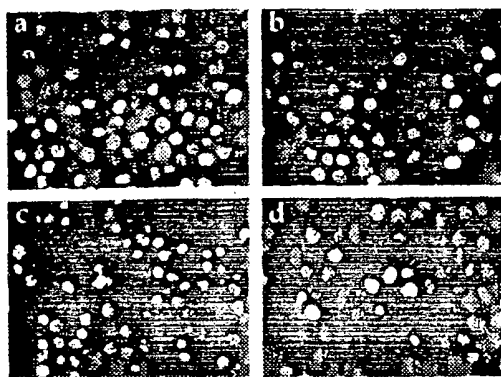
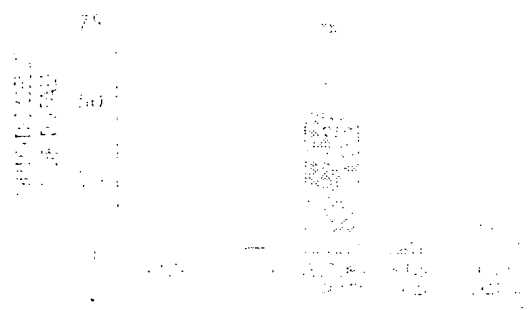


Fig. 31A



42/42



Fig. 32A

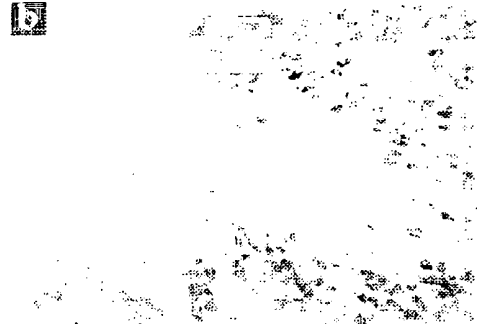


Fig. 32B

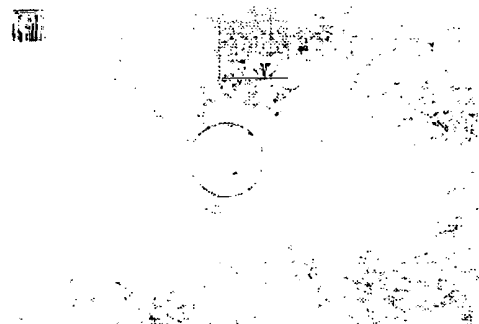


Fig. 32C

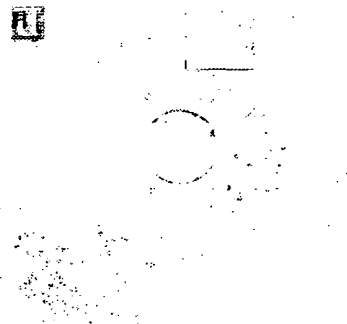


Fig. 32D

SUBSTITUTE SHEET (RULE 26)